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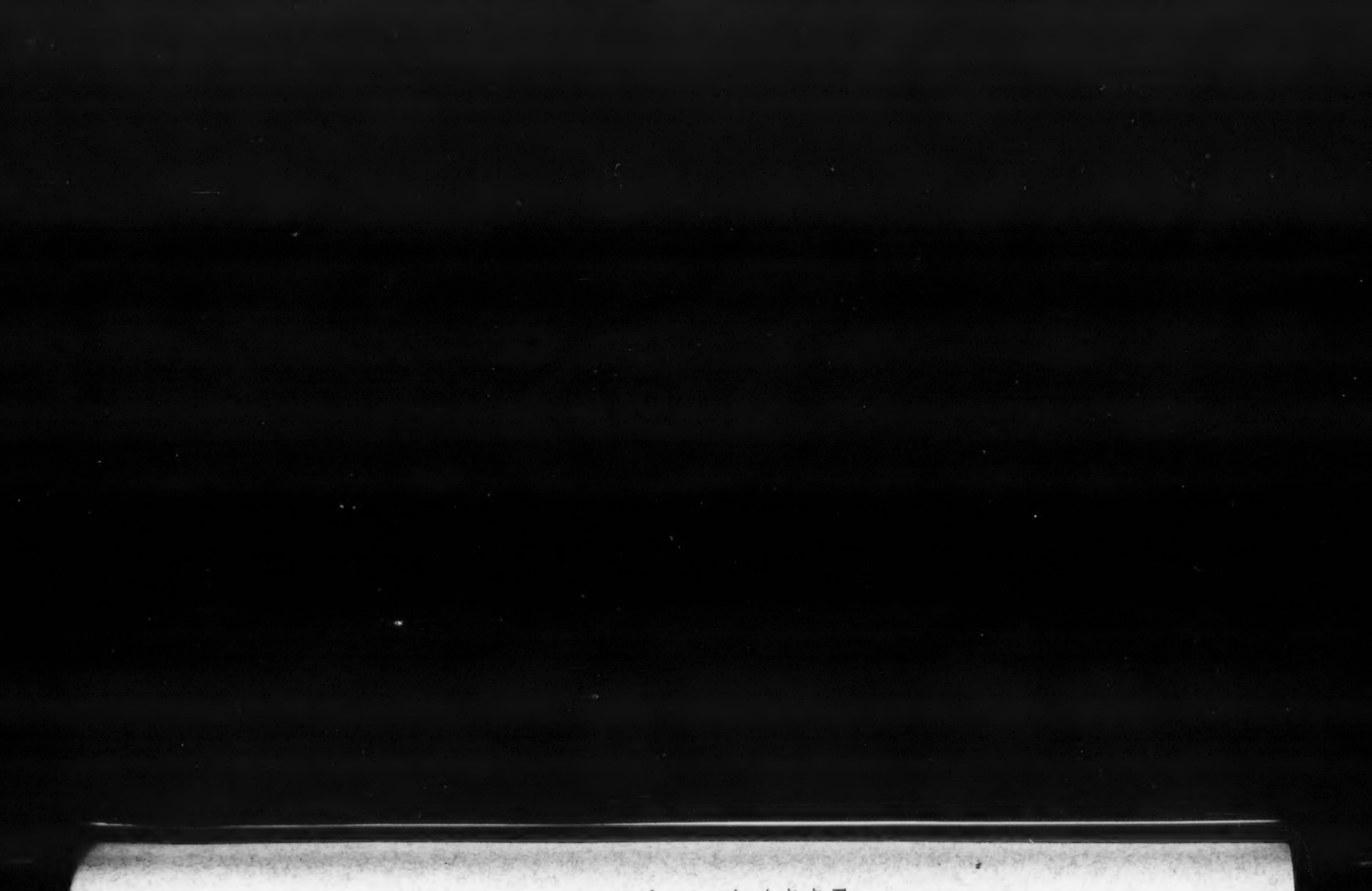

Vol. XLVIII *March, 1925* *No. 3*

BIOLOGICAL BULLETIN

A MODIFICATION OF THE URODELE TESTIS RESULTING FROM GERM-CELL DEGENERATION.

R. R. HUMPHREY.

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Descriptions of the Urodele testis, particularly those of the earlier investigators (von Wittich, '53; Hoffmann,'73, '78; Spengel, '76) frequently contain references to peculiarities of structure, such as lobation, constriction, or reduction in size of caudal or cephalic ends. The significance of such peculiarities was apparently but little understood or appreciated. Although Spengel makes the brief (and correct) statement that these are but the result of the progressive and regressive changes in the testis, no elaboration of this view serves to show whether the statement was more than a speculation.

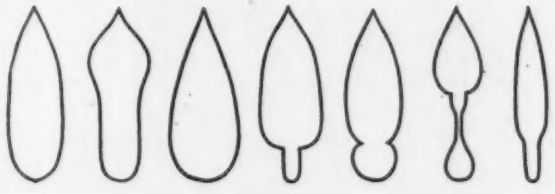
The simplicity of the structural plan of the testis in many Urodeles (the *Plethodons*, *Eurycea*, *Desmognathus*, *Gyrinophilus*) makes the organ particularly susceptible to modifications in form. Of an elongated, cylindrical shape (Fig. 1) it is traversed by a longitudinal collecting duct, more or less central in position, around which the chambers or lobules are arranged in either radial or fan-like fashion, as shown in Fig. 8. The synchronous development of all the cells of the lobule, and the caudo-cephalic order of development and extrusion of the germ cells, in a testis of this type, results in normal seasonal variations that would not otherwise be possible. The earlier emptying of its lobules, for example, reduces the size of the caudal portion of the testis as compared with that territory yet containing spermatozoa (Fig. 2). Later, after the final emptying of the anterior lobules, the inequality in size of the two regions may be quite reversed (Fig. 3) due to the earlier growth, in the caudal lobules, of the spermatocytes for the next cycle. It can be readily appreciated,

too, that an unusual degeneration of germ cells, limited to any one region, would reduce the size of the testis at that point, producing either a slender extension of the organ (Fig. 4) or a constriction between two regions of greater diameter (Fig. 5). In *Desmognathus* and other Urodeles (*Diemyctylus*, *Salamandra*) the peculiar pattern of spermatogenesis ultimately converts the simple type of testis above described into a "multiple testis," that is to say, a structure consisting of from two to five simple testes joined in series by slender intermediate regions containing only the central duct and a few surrounding primary spermatogonia. Figure 6 indicates in outline the form of a "multiple testis" of two lobes. The origin of this peculiar type of testis has been discussed at length in a separate paper (Humphrey, '22). In other species (the *Plethodons*, *Gyrinophilus*, *Eurycea*) a "multiple testis" has never been encountered by the writer in an examination of hundreds of specimens. The testis remains a simple or unit structure, tapering anteriorly, more or less uniform in diameter throughout its greater extent, and ending caudally either in a bluntly rounded fashion (Fig. 1) or as a slender prolongation, variable in length, which I shall designate as the caudal appendage (Figs. 4 and 8). This structure constitutes the subject of the present study.

Such an appendage will be encountered in practically every adult *Plethodon glutinosus* male killed during the summer months. It will be found in a majority of *Gyrinophilus* males, but in only a small percentage of *Eurycea* and *Plethodon cinerius*. Its length varies, even in males of the same species, equal in size, killed at the same time of the year. It may be so short as to be scarcely distinguishable by gross examination, or it may approximate a fourth of the total length of the testis. Its diameter, usually rather uniform throughout, is but a fifth to a third that of the testis immediately anterior to it. The two regions, as a rule, are sharply differentiated as to size, the caudal portion or appendage not increasing perceptibly in diameter towards its junction with the larger "body" of the testis. (The two regions, indeed, may even be separated by a slight constriction.) The caudal appendage would usually differ in appearance, then, from the gradually tapering anterior end of the testis of figs. 1 to 7. It would differ, too, from the similarly tapering posterior end of the

testis in *Ambystoma*, or the bluntly-rounded termination of the organ in other Urodele males lacking an appendage.

To understand the origin and significance of the appendage it is necessary to note its seasonal changes, both in gross appearance and in histological detail. These will be first outlined as they occur in *Plethodon glutinosus*, a species in which the appendage is practically a constant feature in the adult male. It is most conspicuous in animals killed from May to July. Since the body of the testis is then approaching its maximum diameter, due to



FIGS. 1 to 7. A series of diagrams to illustrate various forms of the testis commonly encountered in Urodeles. The cephalic end of the testis is uppermost in each case.

FIG. 1. Elongated, cylindrical type of testis, tapering anteriorly, bluntly rounded posteriorly. Found in the Plethodons, *Eurycea*, and *Gyrinophilus* during winter months, and, in some males of these species, in summer.

FIG. 2. A modification of the testis of Fig. 1 resulting from earlier emptying of the caudal lobules. The anterior lobules still contain spermatozoa. A testis of this form is encountered during the fall or early winter months in the Plethodons and other Urodeles, and is reduced to the type of Fig. 1 by the final emptying of the anterior lobules.

FIG. 3. A modification of the testis of Fig. 1 resulting from earlier development of the germ cells in caudal lobules. Seen as a rule in spring months or in young males in which a smaller proportion of the lobules produce spermatozoa.

FIG. 4. Testis with a caudal reduction ("caudal appendage") resulting from degeneration of germ cells in the caudal lobules of the organ. Found commonly in *Plethodon glutinosus* during the summer months; less common in *Eurycea* and *Plethodon cinerius*.

FIG. 5. Testis with a constriction resulting from localized germ-cell degeneration. Found only occasionally, sometimes as an apparent preliminary condition to that illustrated in Fig. 4.

FIG. 6. "Multiple testis" of *Desmognathus* resulting from delayed regeneration of lobules emptied in each sexual cycle. Spermatozoa for the following cycle are always produced in lobules ahead of those maturing germ cells in the current season.

FIG. 7. Testis of *Gyrinophilus* in summer. In this species the caudal appendage, though structurally similar to that of *Plethodon glutinosus* (Fig. 4) is rendered less conspicuous by the greater length and reduced diameter of the organ as a whole.

the growth and divisions of its spermatocytes, the slender caudal appendage is naturally in obvious contrast as to size (Fig. 4). In the fall, after extrusion of the spermatozoa, the testis proper is greatly reduced in diameter; the appendage, meanwhile, has become considerably enlarged. During the winter, as a result, the testis appears of more nearly uniform diameter throughout, as in Fig. 1, with little or no indication of the caudal reduction so striking in the preceding summer. Nevertheless, in the spring the appendage once more makes its appearance.

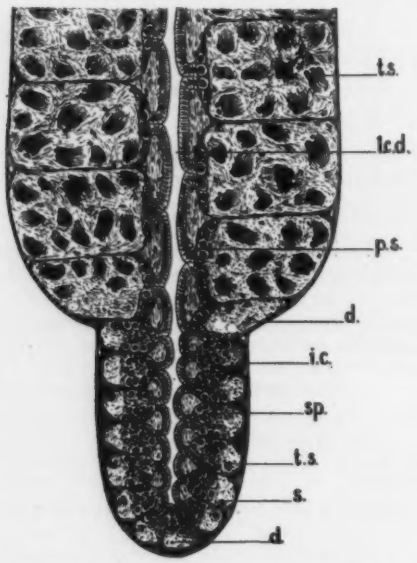


FIG. 8. Caudal third of the testis of *Plethodon glutinosus* in late June. Semi-diagrammatic. The reduction in size of the lobules of the caudal appendage is strikingly shown; but little debris from degenerating cells now remains. Though the body of the testis at this season contains transforming spermatids, the lobules of the appendage show only occasional germ cells other than their apical spermatogonia. *T.s.*, transforming spermatids; *l.c.d.*, longitudinal collecting duct, central in position, surrounded by connective tissue; *p.s.*, primary spermatogonium (residual spermatogonium) of lobule apex; *d.*, nuclear débris from recently degenerating germ cells; *i.c.*, interstitial cells; *sp.*, group of spermatids; *s.*, a primary spermatocyte.

Examined in sections, the appendage of a male killed in early June is found to be traversed by the central collecting duct of the

testis (Fig. 8), around which are lobules, radially arranged as in the testis proper, but of much smaller size. These small lobules contain, at their apices, primary spermatogonia of the usual type. The peripheral portions of the lobules are filled with cells of various types: a few secondary spermatogonia, an occasional growing or dividing spermatocyte, or small group of spermatids, and degenerating germ cells or cell debris among scattered Sertoli cells (see Figs. 8 and 9). The lobules of the testis proper

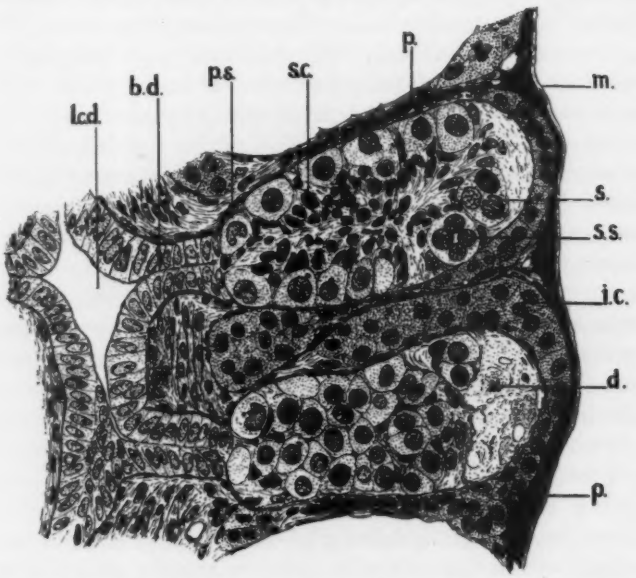


FIG. 9. Two lobules of the caudal appendage of *Plethodon glutinosus* in late June. Camera lucida sketch to show contents of lobule in greater detail. *L.c.d.*, longitudinal collecting duct; *b.d.*, branch of longitudinal duct leading to lobule; *p.s.*, primary spermatogonium of lobule apex; *s.c.*, Sertoli cell (follicle cell); *p.*, pigment cells of tunic and septa; *m.*, mesothelial covering of testis; *s.*, spermatocyte I; *s.s.*, secondary spermatogonium; *i.c.*, interstitial cell sheath around lobule; *d.*, degenerating cells or débris from same.

contain, at the same time of the year, transforming spermatids, dividing spermatocytes, growing spermatocytes, and spermatogonia, in the caudo-cephalic succession characteristic of this and other Urodeles. In accordance with the usual postero-anterior development of the germ cells in these species (spermatogenetic

wave; Kingsbury, '02; Humphrey, '21, '22), the caudal region or appendage would be expected to contain at this time, in addition to its residual primary spermatogonia, only cells further advanced in development than those of the region anterior to it. That is, we should expect to find here only numerous spermatids undergoing transformation, rather than the several sparsely-represented cell types actually encountered.

Later in the season (July, August) it will be found that some of the spermatids in the appendage may have undergone transformation; a very small number of spermatozoa may eventually mature and leave the lobule. This number is as a rule negligible. The slow increase of secondary spermatogonia is the most important change in the lobule of the appendage during the summer; these arise by proliferation of the residual primary spermatogonia of the lobule apex. Such primary spermatogonia always are to be found in the appendage, though all other germ cell stages be absent. The appendage is therefore not a sterile region of the gonad, as is the caudal portion of the testis of certain Teleosts. Germ cells are never entirely lacking.

After the extrusion of the spermatozoa from the testis proper, a prompt regeneration of its emptied lobules is accomplished through the rapid multiplication of their apical spermatogonia. This, indeed, begins some time before the lobules are actually emptied. The development of spermatogonia in the lobules of the caudal appendage now keeps pace with—or, to be exact, precedes—their development in the lobules directly anterior to it. The enlargement of its lobules, of course, explains the disappearance of the appendage as such in animals examined in autumn, the division of the testis into two regions becoming more and more completely obliterated, as lobules throughout the testis become uniformly filled with secondary spermatogonia.

The spermatogonia of the regenerated lobules now begin to undergo the characteristic transformation by which they become spermatocytes of the first order. The latter then enter upon their growth period. Following the rule of progressive caudo-cephalic development, the formation of spermatocytes first occurs in the most caudal lobules, formerly the territory of the appendage. Lobules successively farther and farther cephalad proceed in development after the same manner, until in October

from half to three fourths of the testis contains primary spermatocytes. Meantime, however, in the most caudal lobules these spermatocytes have completed their maturation divisions. Since these too proceed in regular caudocephalic order, it follows that the most caudal portion of the testis finally will contain spermatids. These, in *Plethodon* males killed in late October or in November may be found in as much as a fourth of the extent of the testis, or, on the other hand, in but a few of its most caudal lobules. The onset of cold weather finally checks the further course of spermatogenesis, and few or no maturation divisions then occur until the following spring.

It is in the fate of those spermatids formed in autumn that we find the correct explanation of the caudal appendage seen in the following May or June. For these spermatids, apparently unable to transform, undergo degeneration and resorption, disappearing during the early spring months. The lobules which they occupied now become very much reduced in size, and the caudal portion of the testis, as a result, assumes the proportions of a degenerate appendage of the larger anterior part. In this appendage, as has been mentioned, an occasional spermatocyte may develop (See Fig. 9) and go through its maturation divisions; this may sometimes result in the belated formation of a very small number of spermatozoa. Any extensive development of spermatogonia and spermatocytes in this region, however, is delayed until the time when such a development would occur in preparation for the next spermatogenetic cycle. It is not until late summer or early fall, therefore, that the growth processes in the appendage, now equivalent to those of the lobules anterior to it, obliterate the distinction between the two regions.

A caudal appendage similar to that in *Plethodon glutinosus* is found in practically all *Gyrinophilus* males killed during the summer months. In this species, however, the greater length and reduced diameter of the whole organ (Fig. 7) renders the appendage less conspicuous. Microscopically its structure is comparable to that of the appendage in *Plethodon*, although it shows an apparently greater effort toward compensatory development of germ cells. All the reduced lobules contain a small number of primary spermatocytes, or, later in the season, spermatids or spermatozoa. Because of their small number,

these germ cells do not interfere with the increase of spermatogonia at the central apex of the lobule. In early fall, consequently, spermatogonia are more abundant and farther advanced in development here than in the body of the testis. With the extrusion of the spermatozoa from the latter region, and its consequent reduction in size, the distinction between body and appendage is obliterated, as in *Plethodon*.

The caudal part of the testis, in October and November, always contains spermatocytes in the growth period. Just as in *Plethodon* the germ cells of this region precede in development those of more anterior lobules. In *Gyrinophilus*, however, the spermatocytes do not begin their maturation divisions in the fall. No spermatids appear in a specimen killed on November 19. In *Plethodon glutinosus* the caudal fourth of the testis may contain spermatids as early as November 1.

How, then, does the appendage of *Gyrinophilus* arise? No spermatids are formed in autumn, to degenerate during the winter as in *Plethodon*. Nevertheless the appendage is of almost as constant occurrence in one species as in the other. A clue to its origin in Gyrinophilus is furnished by specimens killed in late March. In these the testis is already greatly reduced in diameter at its posterior end. Here the lobules contain, in addition to their apical spermatogonia, only a very few primary spermatocytes. The majority of these latter cells have completely disappeared; the peripheral portions of the lobules they occupied are now greatly reduced in size and contain only a syncytium of Sertoli cells. Toward the body of the testis, however, the diameter of the appendage increases. Lobules in this region show within the syncytium of Sertoli cells, traces of cell debris and cells far advanced in degeneration. Still farther forward, in lobules of approximately the normal size, the earlier stages of this cell degeneration are to be encountered. Here one finds all intermediate conditions between the small, densely-staining masses characteristic of karyolytic cells in advanced stages, and primary spermatocytes in which the first indication of degeneration is to be seen in a slight contraction of the nucleus and a massing of its chromosomes. Degeneration seems to begin when the spermatocytes are in the midst of their growth period, with the chromosomes already well-defined and definitely oriented

with respect to the centrosomes. In none of the animals killed in either November or March were maturation divisions to be encountered.

It would appear, therefore, that in *Gyrinophilus* a degeneration of primary spermatocytes takes place during the winter months. Since cells of this stage of development are found in autumn only in the caudal part of the testis, it follows that only this region will be reduced in size through their later degeneration and resorption.

In *Eurycea* and in *Plethodon cinerius* the caudal appendage is much less frequently encountered than in *Gyrinophilus* or *Plethodon glutinosus*. In these species, too, it is frequently so small as to be overlooked, except in serial sections of the organ, and, in many cases, is so poorly marked off from the body of the testis that it appears to be merely a caudal tapering of that structure. In its microscopic structure, and in its mode of development it is, however, entirely comparable to the appendage in the forms already considered.

In *Eurycea* males a considerable proliferation of germ cells in the appendage often causes it to resemble a testis in miniature, with the same seriation of developmental stages as occurs in the body of the testis ahead of it. Its germ cells are always retarded in their development, however, as compared with those of the testis proper. For example, in a well-defined appendage of this sort, in mid-August, the caudal lobules contain untransformed spermatids, while the corresponding caudal lobules in the body of the testis are filled with well-developed spermatozoa. Earlier, in July, I have found an appendage filled with spermatocytes I, the body of the testis at the same time containing transforming spermatids throughout its caudal half.

The mode of origin of the appendage in *Eurycea* differs only in details from that described for other species. The primary spermatocytes do not normally develop until the spring months. A male killed in early May, for example, has the caudal third of the testis filled with the so-called synizesis figures characteristic of the early part of their growth period. The development of a caudal appendage through a winter or spring degeneration of spermatids (or growing spermatocytes) is impossible in such an animal. The caudal lobules of the testis in spring show the

probable mode of development of the appendage in *Eurycea*. These lobules contain numerous karyolytic cells, particularly in their peripheral portions. In the most caudal lobules, a syncytium of Sertoli cells, with cell debris and fat droplets in its meshes, indicates the region of earliest degeneration; here the lobules are already greatly reduced in size. More anteriorly fewer degenerations have been completed and the earlier stages of nuclear breakdown are readily observed. Here the lobules are as yet but little reduced in size. An appendage of the testis is therefore not sharply marked off at this time, the entire posterior third of the testis having the form of a short cone.

The germ cells appear to begin degeneration immediately after synizesis, or possibly to degenerate without recovery from this condition. They are stricken, therefore, at the very beginning of the spermatocyte growth period, rather than after it has well started, as in *Gyrinophilus*.

It is worthy of note that the proliferation of spermatogonia to replace degenerated germ cells seems to begin early in May. Since a similar proliferation of spermatogonia is taking place at this same time in the more anterior lobules of the testis, it follows that the appendage, later in the year, will correspond in development with the more anterior rather than the posterior portion of the organ.

The appendage of the testis in *Plethodon cinerius* is well developed in but relatively few males. Frequently it includes only two or three caudal lobules; in such cases it would of course be passed over unobserved in gross examination. The structural features of such lobules, however, warrant classing them with the well-developed appendages of other males.

The development of the appendage may be briefly outlined. In males killed in November or December there has been as yet no development of spermatocytes or spermatids. Yet in animals killed as early as March a caudal appendage may be present. At this time its anterior lobules show karyolytic cells; in the body of the testis immediately adjacent (or even in the same lobule or the same cyst with degenerating cells) are spermatogonia of the last generation, ready to transform into spermatocytes. The germ cells therefore appear to be stricken and degenerate during that critical period of transition from spermatogonium to sperma-

tocyte. In such a period, also, occur the degenerationsdescribed by Kingsbury and Hirsh ('12) in *Desmognathus*. These, however, take place at the end of the spermatogenetic cycle, checking the further development of primary spermatocytes for the season, while the degenerations just described in *Plethodon cinerius* occur at the beginning of the cycle and delay the development of spermatocytes until a later (and presumably more favorable) period. By May the caudal portion of the testis proper contains normal growing spermatocytes, while traces of degenerating cells in the appendage have practically or entirely disappeared. During the remainder of the summer the lobules of the appendage contain only a limited number of spermatogonia. There appears to be no compensatory development of spermatocytes as in *Eurycea* or *Gyrinophilus*, so that no germ cells at all are matured in the appendage. In this respect *Plethodon cinerius* is similar to *Plethodon glutinosus*, in which few if any germ cells come to maturity in the appendage. Its spermatogonia proliferate during the summer and early fall. Their increase in number, and the extrusion of the spermatozoa from the body of the testis, at length obliterate the size difference between the two regions, as in the other species considered.

The appendage of the testis in *Plethodon* and other Urodeles, as above described, is clearly different, both in origin and fate, from the reduced caudal portion of the testis often seen in *Desmognathus* and *Diemyctylus*. The latter structure, in adult males, arises by the emptying of the entire region producing spermatozoa. This, of course, occurs at the end of the spermatogenetic cycle. The emptied lobules are not at once restored by proliferation of their residual primary spermatogonia, which in these species may remain quiescent for several months. The result is the reduction of the emptied portion of the testis to a slender cord. Regeneration of the lobules finally taking place at the caudal end of this cord, an enlargement arises at that point, separated from the testis ahead of it by a slender intervening region in which lobule regeneration has not yet occurred (Fig. 6). In this manner arises the so-called lobed or multiple testis of *Desmognathus* and *Diemyctylus* (Humphrey, '22). A necessary condition for its appearance is delayed lobule regeneration. In the species possessing an appendage, quite the reverse of this

condition obtains. Lobule regeneration proceeds without delay, beginning even before the spermatozoa have left the testis. The spermatogonia of the caudal lobules may even transform to spermatocytes, and these complete their growth and maturation periods, within a few weeks after emptying of the lobules begins. It is this precocity of development, in fact, which results in the formation of a caudal appendage, since cells reaching a certain stage during the fall and winter are fated to degenerate. Though this reduces in size the caudal region of the testis which these germ cells occupied, such reduction is never so extreme as is that of the emptied testis of *Desmognathus*, nor is its regeneration so long delayed. The appendage of *Plethodon*, for example, is always restored to the normal diameter of the testis by the proliferation during the summer of spermatogonia for the next sexual cycle; the emptied region in *Desmognathus* does not begin regeneration for several months longer, and even then, at first, only at its caudal extremity. A part of the emptied region in *Desmognathus* or *Diemyctylus* thus comes to be a slender intermediate cord joining two lobes of a multiple testis. The appendage of *Plethodon*, on the other hand, is annually restored to the normal diameter of the testis proper, and a multiple testis in this species is unknown.

From the preceding outline of its origin and fate in different species, the variations in length and diameter of the true appendage should be easily understood. Its length depends upon the number of caudal lobules in which germ cell degeneration has occurred; its diameter varies with the degree to which the products of degeneration have been resorbed, or the extent of the restoration of its germ cells by proliferation of the residual spermatogonia. Its occasional complete absence in summer months indicates that none of the usual degeneration has occurred in the preceding seasons; its constant absence in fall and winter months is due to the annual development of spermatogonia, a process which is common to both the appendage and the testis proper. This obliterates the structural differences between the two regions, leaving them alike save for the slightly more advanced condition of the germ cells in the territory of the appendage.

In addition to its variations as between species and between

individuals of the same species, the appendage undoubtedly varies in length in the same individual in successive years of its adult life. Environmental conditions differ from year to year; this variation is likely to be reflected in the spermatogenetic processes. If, for example, the climatic conditions of autumn favorable for the maturation divisions of the spermatocytes (in *Plethodon glutinosus*) are continued long beyond their usual period, a greater portion of the testis is filled with spermatids. If, on the other hand, these maturation divisions are checked by the unusually early onset of cold weather, fewer caudal lobules will begin the winter containing spermatids. In the former case, the appendage will be longer, and in the latter case, shorter, when the degeneration of these spermatids has been completed. In some animals, of course, few or no germ cells may reach the critical period of development in the fall months; in this, probably, lies the explanation of the absence of an appendage—a condition frequent in *Eurycea* and *Plethodon cinerius*, though rare in *Gyrinophilus* and *Plethodon glutinosus*. In *Necturus* and *Cryptobranchus* a caudal appendage never develops; a seasonal degeneration of germ cells, of it occurs in these species, is either not limited to one region of the testis or is not sufficiently marked to cause a noticeable reduction in its size.

Degeneration of germ cells in the Urodele testis was early described by Flemming ('87) in *Salamandra*. These cells, it appears, were secondary spermatogonia. From the descriptions of Flemming it would seem that they were not definitely localized in the spermatogenetic cycle or limited to a definite region in the testis. Hermann ('88) describes these same degenerations in greater detail; neither he nor Flemming advance suggestions as to their cause or significance. Drüner ('94) later attempted to demonstrate that these degenerations were caused by the action of a parasite upon the germ-cell nucleus.

The work of Kingsbury and Hirsh ('12) first called attention to the occurrence of such degenerations at a definite point in the spermatogenetic cycle of *Desmognathus*. In this Urodele, the secondary spermatogonia of the "last generation" in each sexual cycle are apparently unable to complete their transformation to spermatocytes and begin the usual spermatocyte growth period. Instead, they undergo degeneration and ultimately disappear.

The polarity of the testis in *Desmognathus*, and the cephalo-caudal seriation of its germ cell stages results in the localization of the degenerating cells in a "zone" near the anterior end of the organ. Cephalad of this, as a "boundary plane," according to Kingsbury and Hirsh, the spermatogenetic processes now lag, no more spermatocytes developing until at the beginning of the next cycle. The degenerations, it is therefore suggested, bear some relation to a regulation of the spermatogenetic processes.

To the writer's knowledge no detailed study of boundary plane degenerations in other Urodeles has ever been reported. The occurrence in *Diemyctylus* of a "boundary plane" comparable to that of *Desmognathus* was noted briefly in a previous paper (Humphrey, '21). The degeneration of spermatogonia in these two species occurs at the same point in the sexual cycle and is limited to a corresponding region of the testis. Similar degenerations would doubtless occur at a comparable time and place in any Urodele showing the same general plan of spermatogenetic pattern (*i.e.*, slow spermatogenetic wave, with delayed lobule regeneration—features resulting in a multiple testis). Spermatogonial degenerations unquestionably occur in Urodeles with a different pattern of spermatogenesis, but in these they are not so definitely localized in the testis and do not affect entire lobules; hence no well-marked "plane" is established.

Though "boundary plane" and other spermatogonial degenerations are possibly associated with a regulation of the spermatogenetic process, it seems unlikely that the degenerations described in this paper have any such significance. They occur at the opposite end of the testis, and—so to speak—at the opposite end of the spermatogenetic cycle. The cells that suffer degeneration are not the last generation of a season's output, but the first. They in each case are most advanced in development of any cells in the testis at the time. They are precociously developed—in some cases several months ahead of the similar cells privileged to go on to complete maturity. Spermatids formed in May in *Plethodon glutinosus*, for example, transform into functional spermatozoa, but spermatids developed in the preceding November usually undergo degeneration before spring. Winter, in this case, comes on after the beginning of a new spermatogenetic cycle; certain cells apparently have reached

a point in development beyond which, under the now unfavorable conditions, further progressive changes seem inhibited. Nor can these cells remain in a resting condition until the advent of spring. Their degeneration and resorption from the caudal lobules of the testis follows, with the results already pointed out.

Strangely enough, the point in development at which the cells are stricken varies with the species. There is, apparently, no one "critical period" common to the four species studied. The four agree in that the germ cells suffering degeneration are in each case those most advanced in development, located in the most caudal lobules of the testis. In *Plethodon glutinosus* these cells are spermatids. Spermatocytes in their growth period are not affected. Yet it is in this latter stage that the germ cells of *Gyrinophilus* begin degeneration. Rather curiously, those germ cells of both *Gyrinophilus* and *Plethodon* which chance to be transforming from spermatogonia to spermatocytes remain unaffected. This transformation period, it will be recalled, is that in which the "boundary plane" degenerations always begin. It is the cells of this stage of development which degenerate in *Eurycea* and *Plethodon cinerius* to produce the caudal appendage. The absence of such an appendage in numerous males of these two species might be assumed to be due either to an absence of cells at the critical stage, or to an increased ability of these cells to survive unfavorable conditions. That the appendage is seldom absent in *Gyrinophilus* and *Plethodon glutinosus* is evidence of the constancy with which germ cells reaching more advanced stages degenerate.

So far as may be perceived, there is nothing inherent in their caudal position in the testis which should cause germ cells to degenerate. This region is apparently as well vascularized as any portion of the testis; its germ cells in autumn show no indication of interference with their development, being always somewhat in advance of those more anteriorly situated. Nor do germ cells of *all* stages degenerate here. The primary spermatogonia always remain unaffected, and later restore the lobules of the appendage through their proliferation. The degeneration, in short, is clearly correlated with the more advanced stage of development of the cells involved, only these cells being unable to survive when conditions become unfavorable. Such un-

favorable conditions, of course, are imposed upon all cells of the testis alike, without reference to their position in the organ.

That cells in more advanced stages of spermatogenesis are least able to withstand unfavorable conditions has been demonstrated experimentally in other animals. Siperstein ('21), for example, has found that in adult white rats subjected to inanition, the more advanced stages of the germ cells are the first to undergo degeneration. Moore ('24), in his study of testis transplants recovered after one to seven months, points out that some influence "prevents the building up of a completed epithelium with differentiated spermatozoa; instead the cells near the stage of differentiation either degenerate in place or are cast off into the lumen of the tubule." In his report on the changes in testes rendered cryptorchid by operation, Moore further states that spermatozoa rarely are present in the tubules seven days after operation; later the remaining cells of the germinal epithelium disappear, the cells persisting longest being those at the periphery of the tubules: *i.e.*, the spermatogonia. It would appear, therefore, that in mammals, as in amphibia, the cells first affected by departure from the normal conditions are those most advanced in development. In the four Urodeles discussed in the preceding pages, conditions become but slightly unfavorable, and only certain of the most advanced cells are affected. A condition comparable to that obtained by Moore in mammals, may, however, be obtained in Urodeles by subjecting the males to inanition over a considerable period of time. The entire testis, in such animals, may be reduced to a slender cord in which no germ cells save primary spermatogonia remain. The lobules as such may entirely disappear. This condition is somewhat comparable to the extreme reduction of the mammalian testis in cryptorchidism, save that in the mammal the reduced tubules are persistent.

In no Urodele examined does germ-cell degeneration commonly reduce more than a fourth of the testis to an appendage. One of even this proportionate length is unusual. It is clear that animals with an excessive degeneration of germ cells would produce fewer mature spermatozoa than males not showing this peculiarity. Such males would probably leave fewer offspring than the male producing a greater number of functional germ

cells. Hence an appendage of excessive length would tend to disappear in the course of phylogeny, the strains surviving being those in which only a more limited degeneration occurs.

In one male however, an unusual type of appendage, fully half the length of the testis has been noted. This male, a *Gyrinophilus* freshly captured on August 15, is of large size, and is possibly a senile individual or one unfavorably affected by disease or other factors. At any rate, the spermatids in the entire caudal half of the testis have failed to transform, degenerating as they developed. This degeneration, it may be noted, is of cells more advanced in development than those whose degeneration in winter ordinarily gives rise to the appendage in this species. The growing spermatocytes, though unable to survive winter conditions, actually complete their growth period and maturation divisions in this specimen, only to have the resulting spermatids destroyed by subsequent degeneration.

Another unusual type of appendage was found in a *Eurycea* male captured in early July and kept in the laboratory for a period of about ten days. The oldest spermatids in this male were probably beginning their transformation at the time of its capture, since, ten days later, developing spermatozoa were present in a few of the most caudal lobules of the testis. Apparently only those spermatids having reached a certain stage of transformation before the period of captivity began were able to continue their normal differentiation, since in lobules more anterior in position, in which the spermatids would have been less advanced, their continued development had become abnormal in character. In these lobules, as a result, various bizarre types of spermatozoa were present, including cells similar in form to the mammalian spermatozoön. In still more anterior lobules the younger spermatids had disappeared completely, probably without ever beginning transformation; cephalad of this region were lobules in which the spermatid degeneration was still going on. Younger stages (spermatogonia, growing and dividing spermatocytes) show no unusual features. Apparently those germ cells most susceptible to environmental effect are those most advanced in development—at least up to a certain stage in spermatid transformation. After this stage the cells are more resistant, as evidenced by the normal spermatozoa in the most

caudal lobules of this male. Fully mature spermatozoa, of course, would probably be even less susceptible than those in transformation. Their survival of the winter in the testes of numerous Urodeles (*Diemyctylus*, *Necturus*, *Ambystoma*, etc.) would bear out this hypothesis.

Unusual types of appendage, such as the two above discussed, further indicate that the similar structure found under usual or normal conditions is but a result of the action of environmental factors upon the spermatogenetic processes. The caudal appendage of such general occurrence is no indication of a functional reduction of the testis in phylogeny or ontogeny, nor does it represent a structural adaptation for a changed function, as do sterile portions of the gonads in certain Teleosts. Its occurrence is limited to testes of a simple type, in which the germ-cell stages occur in caudo-cephalic succession, and cells of any one region are all in approximately the same stage of development. Under such conditions, germ-cell degeneration limited to cells of a particular type readily affects the form of the testis in a particular limited region. In a testis in which every tubule contains cells of numerous types (as in anuran, reptile, bird, mammal) such modifications of the organ are of course impossible.

Summary.

1. The testes of several species of Urodeles frequently possess a slender caudal extension or appendage suggestive of a functional reduction of the organ.

2. This caudal appendage is present only during the summer months. It disappears in autumn, to reappear in the following spring.

3. Study of its cyclic changes indicates that its appearance is due to degeneration of the germ cells of the more caudal lobules of the testis.

4. The germ-cell degeneration is interpreted as resulting from the unfavorable conditions of winter; the cells most advanced in development are apparently the most susceptible and they alone degenerate.

5. The caudo-cephalic order of development of the germ cells results in the localization of the most advanced stages in the caudal end of the testis; their degeneration reduces this region to a slender caudal appendage.

6. The development of spermatogonia for the next spermatogenetic cycle occurs at the normal time in the appendage, increasing its size to that of the "body" of the testis; this explains the annual disappearance of the appendage in species possessing it.

7. The development of an appendage of the type here described is possible only in certain Urodeles with a testis of simple type and marked polarity. It is of almost constant occurrence in *Gyrinophilus* and *Plethodon glutinosus*.[1]

Postscript.—Since the foregoing paper was placed in the hands of the editor, a number of Urodele males captured in the spring and summer of 1924 have been examined. These animals, particularly in the case of one species, show a very striking difference from those of the four preceding years. While among twenty-two adult *Plethodon glutinosus* males captured between January and September in 1920, '21, '22, and '23, only one lacks the characteristic appendage above described, not one of the nine taken in 1924 possesses this structure. In those males captured earliest in the season (May and early June, 1924), normal spermatocytes still occupy that caudal portion of the testis in which spermatids are commonly found to have been formed in early autumn. Evidently few or no maturation divisions occurred in the fall of 1923, and as a result, few or no spermatids were present to degenerate during the winter of 1923–24. Absence of the appendage in 1924 is the natural result of such retarded spermatogenesis.

The failure of the maturation divisions to begin in the fall of 1923 is undoubtedly a consequence of climatic conditions obtaining during the spring and possibly also the fall of that year. Weather bureau records at Ithaca show that the average temperature for May, 1923, was 4.8 degrees below that of the normal (average of 30 years). *Plethodon* males taken in July, 1923, show spermatogenesis retarded by from two to four weeks as compared with males taken in other years,—a retardation presumably due to the lowered temperature above noted. This retardation apparent in July would undoubtedly delay comple-

[1] The writer wishes to acknowledge the helpful suggestions and criticism of Dr. B. F. Kingsbury of Cornell University, in whose laboratory the investigation was begun.

tion of the current spermatogenetic cycle and postpone the beginning of the cycle following. At any rate, no maturation divisions occurred in the fall of 1923 in this species. The spermatocytes present in the testis were either delayed in their early development, or their maturation divisions were later prevented by lowered October temperature. Both factors might conceivably be involved in effecting the end result, the elimination of the usual autumnal maturation. Since only spermatids undergo degeneration during winter in *Plethodon glutinosus*, no appendage appeared in the following season.

Of the other Urodele species developing an appendage, *Plethodon cinerius* shows an effect comparable to that in *Plethodon glutinosus*. In *Eurycea* the appendage was found during 1924 much as in preceding years. No *Gyrinophilus* males were examined.

The complete absence of the appendage here reported strikingly supports the writer's statement that it is a structure due to environmental effects upon spermatogenetic processes, and as such, may vary from year to year even in the same male.

LITERATURE CITED.

Drüner, L.

'94 Beiträge zur Kenntnis der Kern- und Zellen-degeneration und ihre Ursache. Jenaische Zeitschr. f. Naturw., Bd. 28.

Flemming, W.

'87 Neue Beiträge zur Kenntnis der Zelle. Arch. f. mikr. Anat. Th. I. Bd. 29

Hermann, L.

'88 Ueber regressive Metamorphosen des Zellkerns. Anat. Anz., Bd. 3.

Hoffmann, C. K.

'73–78 Dr. H. G. Bronn's Klassen und Ordnungen des Thierreiches. 6 Bd., 2 Heft.

Humphrey, R. R.

'21 The Interstitial Cells of the Urodele Testis. Am. Jour. Anat., Vol. 29.

Humphrey, R. R.

'22 The Multiple Testis in Urodeles. BIOL. BULL., Vol. 43.

Kingsbury, B. F.

'02 The Spermatogenesis of *Desmognathus fusca*. Am. Jour. Anat., Vol. 1.

Kingsbury, B. F., and Hirsh, Pauline E.

'12 The Degenerations in the Secondary Spermatogonia of *Desmognathus fusca*. Jour. Morph., Vol. 23.

Moore, C. R.

'24 The Behavior of the Germinal Epithelium in Testis Grafts and in Experimental Cryptorchid Testes. Science, N. S., Vol. 59, P. 41.

Siperstein, D. M.

'21 The Effects of Acute and Chronic Inanition upon the Development and Structure of the Testis in the Albino Rat. Anat. Rec., Vol. 20.

Spengel, J. W.

'76 Das Urogenitalsystem der Amphibien. I. Thiel. Der Anatomische Bau des Urogenitalsystems. Arbeiten aus dem zoölog.-zoötom. Institut in Würzburg. 3 Bd., 1 Heft.

von Wittich.

'53 Beiträge zur morphologischen und histologischen Entwicklung der Harn- und Geschlechts-werkzeuge der nackten Amphibien. Zeitsch. für wissensch. Zoöl., 4 Bd.

AN APTEROUS MUTATION IN *BRUCHUS*.[1]

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There appeared in my cultures of *Bruchus quadrimaculatus* Fabr., the "four-spotted cowpea-weevil," a mutation entirely void of wings. It was discovered at Cold Spring Harbor on August 5, 1918, in seven females. The wild stocks from which these wingless females emerged had passed through twenty-five generations of selective breeding. This mutation could have only come from a single pair. The culture was obtained from Raleigh, North Carolina, on January 18, 1916, and had been examined daily for various mutations. It is known therefore that at no previous time had any such apterous beetles appeared. During the eight years of breeding this insect no more such mutations have been observed among the thousands of weevils examined. The appearance of this Bruchid's wings is not unlike that described by Metz ('14) for apterous *Drosophila*. Even though the cultures of this apterous insect died out, due possibly to some genital defect, there are some genetic interpretations of value which may be made.

This apterous beetle was first discovered in seven females which made their appearance during two days from one wild culture bottle. Because they were the first mutants observed in such great numbers, they were at first thought considered abnormalities or freaks rather than mutations. But when they again emerged in the F_2 from apterous females mated with wild males from another North Carolina culture, it was evident that this trait was a recessive one.

Concerning the origin of mutations in *Drosophila* Lancefield ('18) states that if a mutation occurred in the early oögonial stages several with the new gene should appear. This answers the question why several of the mutants could have been revealed. The fact that they were all of one sex might add evidence to such

[1] Contribution from the Zoölogical Laboratory of the University of Oklahoma, Second Series, No. 46.

a conclusion. However, in the tests which follow, it is demonstrated that the genes are carried by both sexes, but are visible only as phenotypes in the females. The trait is therefore sex limited. If this is true, the character could have originated possibly in the male previously but remained unseen. A recessive mutation, like apterous, could not be seen in either sex until the second generation after its mutation. This is the probable origin and accounts for the seven apterous insects discovered in the same generation.

Each apterous *Bruchus* was mated to a normal winged brother from a second North Carolina culture. Seven pairs in all were mated (Table I.), but only two produced progeny, pairs 2 and 6. Pair 2 in the F_1 generation produced 4 normal winged females and 5 normal winged males. These were inbred as a mass culture in the F_2, giving 13 winged females, 2 apterous females, and 11 winged males. The two apterous females were mated with an F_2 winged male from this same fraternity but no offspring were found. Pair 6 was the other of the seven apterous insects which had progeny. They produced in the F_1 9 winged females and 8 winged males. These F_1 beetles were inbred as a mass culture. Their progeny consisted of 9 winged females, 3 apterous females, and 12 winged males in the F_2. Each of these three F_2 apterous beetles was mated with a male from the same fraternity, but no progeny was found from any pair of the three. Again from this same F_2 fraternity 6 winged females were bred to five winged males. They produced in the F_3 7 winged females 3 apterous females, and 9 winged males. Another winged pair from the F_2 gave five winged females and two winged males in the F_3. The last two of the F_2 females were mated with the last 5 winged males of this same fraternity. They produced in the F_3 6 winged females, 4 apterous females, and 12 winged males. Each of these four, F_3, apterous was bred with a winged male from a wild culture, the same North Carolina stock but only two pairs gave offspring in a mass culture consisting of 8 winged females and 7 winged males in the F_1 generation. These bred in mass produced 25 winged females, 4 apterous females, and 30 winged males. This apterous trait came to an end when each of these four wingless females were each mated with a male from a North Carolina culture from the experiment table. The apterous

insects could possibly have been saved by entirely mass cultures, crossed with stocks having low sterility or high fecundity.

This rather incomplete data shows that apterous is a simple recessive. That the majority of apterous females did not produce offspring might be correlated with the idea that such females have genital openings too small for the male copulatory organ to enter. Another apparent fact is that sterility was manifested throughout, which might be interpreted as being due to the stock from North Carolina in which apterous originated and in which all apterous matings were made.

The wild North Carolina cultures, in which the apterous insects were discovered, were dead by November. They always produced a less number of progeny than any other wild stock since sterile pairs were always common; however, the Texas wild cultures continually produced a great number of offspring. All wild stocks were kept from generation to generation by mating one or five pairs each time. More pairs mated possibly would have been sufficient to have preserved the North Carolina stocks alive. This sterility could have been eliminated by crossing apterous to the Texas cultures instead of the one in which it arose, but was the only one present at this time. This relative infecundity, further, accounts for the low number of offspring discovered in these experiments. It is unknown to which autosome apterous or sterility was linked, because no crossover tests were possible.

The average number of progeny produced per pair is the lowest observed. Table I. for 50 pairs mated gives 198 offspring, which is for each pair an approximate 1:4 ratio. For a comparative conception the following ratios are summarized from another set of experiments: a 1:5 ratio for pure lines of white body color and black spots on elytra, the $R^{w}s$ cultures; a 1:38 ratio for red body color and black spots on the elytra, the Rs cultures; a 1:50 ratio for red body color and red spots on elytra, the RS cultures; a 1:46 ratio for black body color and black spots on elytra, the $R^{b}s$ culture; and, lastly, one wild culture, rs, gave a 1:52 ratio. This wild culture is the highest of all in giving a greater average of adults from each pair bred.

It is about thirteen times more prolific than the apterous wild stock from North Carolina. Aside from this extremely low

number of progeny manifested in apterous matings, it is very probable that there must be some defect of the genital organs of the apterous females because (Table I.) of the nineteen pairs of

TABLE I.

COMPLETE BREEDING RECORDS FOR APTEROUS.

The F_1 offspring are indicated below as all winged with no apterous.
The F_2 progeny show both winged and apterous.

Mating No.	Pairs Mated Apterous Females with Winged Males.	Offspring.			Total.
		Females.		Males, Winged.	
		Winged.	Apterous.		
1.....	1	0	0	0	0
3.....	1	0	0	0	0
4.....	1	0	0	0	0
5.....	1	0	0	0	0
7.....	1	0	0	0	0
2.....	1	4	0	5	9
2.1.....	4*	13	2	11	26
2.11.....	1	0	0	0	0
2.12.....	1	0	0	0	0
6.....	1	9	0	8	17
6.1.....	9*	9	3	12	24
6.11.....	1	0	0	0	0
6.12.....	1	0	0	0	0
6.13.....	1	0	0	0	0
6.14.....	6*	7	3	9	19
6.15.....	1*	5	0	2	7
6.16.....	2*	6	4	12	22
6.161.....	1	0	0	0	0
6.162.....	1	0	0	0	0
6.163.....	2	8	0	7	15
6.1631.....	8*	25	4	30	59
6.16311.....	1	0	0	0	0
6.16312.....	1	0	0	0	0
6.16313.....	1	0	0	0	0
Totals.....	50	86	16	96	198

* Indicates all winged pairs in F_1 with F_2 offspring.

apterous females mated only four produced offspring. Two of these were single pairs of the first seven apterous mutations: Pairs 2 and 6 respectively. The other two apterous pairs were bred as mass cultures and they produced offspring. Aside from this apparent sterility, it is possible that the genitalia of the female is too small for the male to copulate with the female in every instance.

Metz ('14) states that low viability of the apterous flies causes more normals in the F_2. This is also true in *Bruchus* since there are more normals in the F_2, which consists of a total of 65 winged females, 16 apterous females, and 76 winged males. The ratio is approximately a 4: 1 instead of a 3: 1 ratio, because in such matings there would naturally be more pure dominants concerned. Aside from this the males do not show the trait which would also tend to make the ratios less normal.

Metz ('14) again, with reference to apterous flies being sex linked, states that the absence of apterous flies in the F_1 indicates that the character is not sex linked. It is true in *Bruchus* that the F_1 produces no apterous beetles, but this is not a safe criterion in *Bruchus*, because all males are always winged, regardless of their transmitting the apterous trait, that they may carry. The F_2 is the ideal place to determine it. All of the P_1 matings were apterous females with winged males. The F_2 if sex linked should give half females apterous and half winged or the reciprocal cross would give all females winged. This is not true for apterous in *Bruchus*, because the ratio approximates a 3: 1 ratio in the F_2. The character is therefore not sex linked.

In conclusion, apterous is a recessive trait seen only in the females. It has a high degree of sterility possibly from the North Carolina stock in which it originated. Lastly, it has a lower viability than the normal.

BIBLIOGRAPHY.

Lancefield, D. E.

'18 Three Mutations in Previously Known Loci. Amer. Nat., Vol. LII., p. 264–269.

Metz, Charles W.

'14 An Apterous *Drosophila* and its Genetic Behavior. Amer. Nat., Vol. XLVIII., p. 675–692.

ASEXUAL REPRODUCTION IN THE STARFISH, *SCLERASTERIAS.*

W. K. FISHER.[1]

In sea stars, self-division involving the disk occurs much more rarely than autotomy of rays only. The first condition, termed fissiparity, is a form of asexual propagation and in the Asteriidæ is characteristic of three genera, namely, *Coscinasterias* (both the *calamaria* type and the *tenuispina* type), *Sclerasterias*, and *Stephanasterias*. In the last genus asexual reproduction appears to be fully as important as gametic. In *Linckia*, belonging to a different order, an entire animal may be regenerated from a cast-off ray. The curious comet forms originate in this manner.[2]

In *Coscinasterias* and *Stephanasterias* fissiparity persists in adult life, but in *Sclerasterias* [3] it is strictly confined to a very immature stage, in which the number of rays is predominantly *six*, whereas in the adult it is five. Furthermore these young differ so

[1] From the Hopkins Marine Station of Stanford University.

[2] For figures see Fisher, "Asteroidea of the North Pacific," Bull. 76, U. S. Nat. Mus., 1911, plate 48, Figs. 1 and 2.

[3] *Sclerasterias euplecta* is figured in Fisher, "The Starfishes of the Hawaiian Islands," U. S. Fish. Comm. Bull. for 1903, part 3, plate 42, Figs. 1 to 4. Figure 3 is in the intermediate stage. The closely related *S. hypacantha* is figured in Fisher, "Starfishes of the Philippine Seas," Bull. 100, U. S. Nat. Mus., 1919, pl. 141, Figs. 2, 3.

The species of *Sclerasterias* resemble *Marthasterias glacialis*, the well-known European sea star. The genus is a fairly homogeneous group of thirteen known species, related to *Coscinasterias*. Its type, *S. guernei*, was supposed to be a highly peculiar small species but proves on examination to be immature and congeneric with *Eustolasterias* Fisher, a name of later application. From among the thirteen species may be mentioned: *S. alexandri* (Ludwig), Bay of Panama; *S. contorta* (Perrier), Florida to Barbados; *S. euplecta* (Fisher), Hawaiian Islands; *S. eustyla* (Sladen), Tristan da Cunha; *S. guernei* Perrier, genotype, Bay of Biscay; *S. mollis* (Hutton), New Zealand; *S. tanneri* (Verrill), Atlantic coast of United States; *S. heteropæs* Fisher, new species, Monterey Bay, California.

Perrier states that his genus *Lytaster* is fissiparous. I can confirm this from an examination of the types, at Paris. But the types are only very immature specimens of *Coscinasterias tenuispina*.

For a synopsis of the family Asteriidæ, see Fisher, *Annals and Mag. of Nat. Hist.* (9), Vol. 12, 1923, pp. 247, 595.

markedly from the adult [4] that they have been described as of a different genus. I have found this condition in three species: *Sclerasterias euplecta* (Fisher), *S. heteropæs*, new species, and *S. alexandri* (Ludwig). The young of the last was described by Ludwig as *Hydrasterias diomedeæ*, while *Hydrasterias richardi* (Perrier) is the fissiparous phase of an unknown adult.

In this stage the sea stars actively divide by splitting into equal halves. The number of these divisions is not known. In one instance, in *S. euplecta*, a division has taken place nearly at right angles to the plane of the prior fission. Since adult *Sclerasterias* are almost invariably five-rayed and the majority of the young are six-rayed, an arm is lost somewhere in the process—probably at the last division. These dividing young range in size from R 8 mm.[5] to R 20 mm., the latter being unusually large. R 15 mm. is nearer the normal maximum size of fissiparous individuals.

The species of *Sclerasterias* live in deep water, usually among rocks. All the specimens of fissiparous young which I have seen were taken by means of hempen tangles. The data for the following notes are admittedly incomplete. There is every reason to believe, however, that many years will pass before more material is forthcoming.

Of the thirty-six young of *S. euplecta* examined, nine, or one fourth, have five rays. None of these show signs of fissiparity although two have lost individual rays. All of the twenty-six fissiparous specimens have six rays. Of these six rays, three are usually smaller and represent the regenerating half.

In *S. heteropæs*, fourteen of the young have six rays and only one has five rays. Twelve out of these fourteen six-rayed specimens have four madreporites symmetrically placed, with two on either side of the plane of fission (the two opposite interradii through which the disk splits being without them). Thus each half, after fission, has 2 madreporites—one on either side of the

[4] It is not essential to introduce data to prove that these specimens are really young *Sclerasterias*, although there is ample evidence. In the fissiparous stage the crossed pedicellariae (which are like those of the adult) are not concentrated in circumspinal wreaths as in the adult, but are scattered between the spinelets. The latter are short, uniform, and two to four on each median radial plate and two or three on each superomarginal plate. In the adult only each alternate plate of the two series mentioned carries a single spine. The extra spines are absorbed.

[5] *R* is the distance between the center of disk and tip of ray—the major radius.

central ray of the triad (Figs. 1–3). The exceptions are a tiny symmetrical specimen with R 7.5 mm., on which I can find only one madreporite; and a specimen with 2 pores on the regenerating

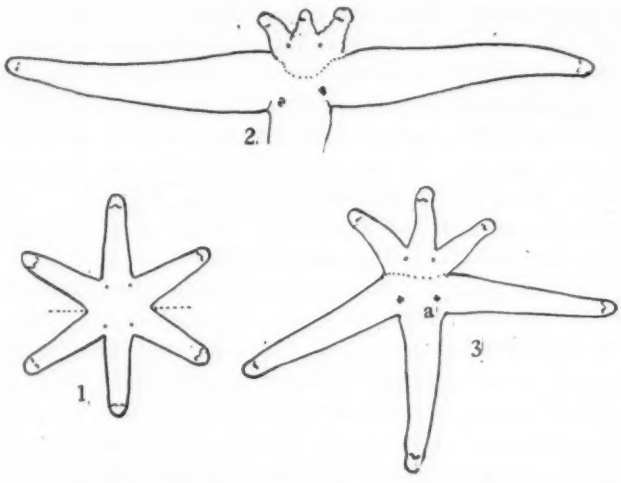


FIG. 1. *Sclerasterias euplecta.* The smallest symmetrical six-rayed specimen *R* 5.5 mm. The dotted lines indicate plane of cleavage. The four madreporites are indicated by dots; × 3.

FIG. 2. *Sclerasterias heteropæs.* Individual regenerating three new rays and two new madreporites; × 3.

FIG. 3. Same; a more advanced stage. In one specimen similar to this the madreporite *a* is lacking; × 3.

half and only one on the original half (Fig. 3). *S. euplecta* has three or four madreporites in regenerating material where the new rays are large enough to have developed pores.

The only five-rayed specimen of *S. heteropæs* has two madreporites in nearly opposite interradii. Of the nine five-rayed examples of *S. euplecta*, eight have one madreporite and one has three madreporites (which are smaller than the single madreporite of the other five-rayed specimens).

It is clear that active fissiparity is correlated with six rays and with usually four symmetrically placed madreporites; for none of the five-rayed examples shows evidence of having split through the disk. In these only separate rays have been shed as in

ordinary autotomy, the disk remaining entire with the five oral angles uninjured. In fissiparity two opposite oral angles are split neatly in twain.

The location of the madreporites with reference to the plane of splitting would provide two directly opposed "physiologically anterior" points (Cole) and would thus automatically favor an equal splitting of the disk. Crozier (20) regards the multiplication of madreporites at separated points on the disk of *Coscinasterias tenuispina* as furnishing an assurance that portions of the body separated by autotomy will each be provided with a madreporic canal. This seems reasonable. However, a large, non-fissiparous species, *Acanthaster planci*, with upward of sixteen rays has four to eight madreporites.

The utility of several madreporites in fissiparous species would appear to be clear. But as to origin, it is not evident in *Sclerasterias* that the extra madreporites are solely post-larval developments as a preparation for fission. Furthermore we have a transitory post-larval hexamerous symmetry to account for in a characteristically pentamerous genus. The six-rayed young with four madreporites may have descended from larvæ with four hydropores. If so it is likely that we have in nature the sort of hydropore duplication reported by Newman in laboratory cultures of *Patiria miniata* (Newman 21, 21a). This physiological twinning in the larva may be here a normal precursor to a subsequent post-larval "untwinning," by which the six-rayed, four-pored, fissiparous young becomes a five-rayed, non-fissiparous adult with one madreporite. [I have specimens showing this last stage, *before* the spines and pedicellariæ have assumed the fully adult reduction and concentration.] In other words some of the incentive to splitting may be due to a sort of physiological duality locked up in the young with six rays. The five-rayed young with one madreporite would naturally be derived from larvæ with one hydropore. Possibly the five-rayed young with two and three madreporites are descended from incompletely twinned larvæ; or again, they may already have accomplished the reduction division without showing outward signs.

Although the mechanics behind this curious condition are as yet material for speculation, the phenomenon itself seems to produce a fairly definite asexual generation following close on the

heels of the larval stage. This asexual mode of reproduction is associated with an abnormal symmetry for the genus.

LITERATURE.

Crozier, W. J.

'20 Notes on some Problems of Adaptation. 2. On the temporal Relations of Asexual Propagation and Gametic Reproduction in *Coscinasterias tenuispina*, etc. BIOL. BULL., Vol. 39, 1920, pp. 122–128.

Newman, H. H.

'21 On the Occurrence of Paired Madreporic Pores and Pore-canals in the advanced *Bipinnaria* Larvæ of *Asterina* (*Patiria*) *miniata*, etc. BIOL. BULL., Vol. 40, pp. 118–125.

'21*a* The Experimental Production of Twins and Double Monsters in Larvæ of the Starfish *Patiria miniata*, etc. Jour. Exper. Zoöl., Vol. 33, pp. 321–352.

THE AXIAL GRADIENTS IN HYDROZOA.

VII. Modification of Development through Differential Susceptibility.

C. M. CHILD.

From the Hull Zoölogical Laboratory, the University of Chicago.

In the preceding paper of this series the physiological analysis of embryonic development in certain species of hydrozoa was begun with a study of the physiological gradients and their changes during the usual course of development (Child, '25). The present paper is a continuation of this analysis and is concerned with the modification of development resulting from the differential susceptibility to various agents of different levels of the polar gradient or gradients.

Material and Method.

Developmental stages of the leptomedusa, *Phialidium gregarium*, abundant in Puget Sound, constituted the chief experimental material. Since the medusæ shed eggs or sperm more or less continuously early developmental stages are readily obtained by keeping the sexually mature medusæ in large containers for a time and then collecting the embryonic stages from the bottom. Developmental material obtained in this way shows a certain range of stages, depending on the length of time the medusæ are kept in the container, but by using large numbers of medusæ sufficient material for one or more experimental series can usually be obtained within a few hours. With this procedure the stages range from newly fertilized eggs to more or less advanced cleavage or early blastulæ and still greater uniformity can of course be obtained by sorting the stages, but this is unnecessary for most purposes. A few experiments were performed with the early planulæ of *Gonothyræa clarkii* after their emergence from the gonophores.

The experimental procedure consisted in the exposure of the developmental stages to concentrations of agents which had been

found by preliminary experiment to inhibit development to a greater or less degree but not to be directly lethal. Agents and indicated concentrations used are as follows: KCN, *m*/20000, *m*/25000, *m*/50000; HCl, pH 6.8 −, pH 7.2, pH 7.3, pH 7.4 +;[1] LiCl, *m*/25, *m*/50, *m*/80, *m*/160; ethyl urethane, *m*/25 and very dilute neutral red. All solutions were made up in sea water and the concentrations given are those indicated by amount of agent and volume of water used. For experiment finger bowls holding some 400 cc. were used. These could be brought under a binocular or even under low power of a compound microscope without removal or disturbance of the developing material. When volatile agents were used bowls with an edge giving good contact with a glass plate were selected, being completely filled with the solution and covered with the plate so as to exclude air. Atmospheric humidity being high, there was practically no loss of water under these conditions and certainly no appreciable loss of the agent since renewal was usually made daily.

The variations in experimental procedure may be grouped as follows: (*a*) continuous exposure to a certain concentration beginning with the earliest stages obtainable; (*b*) temporary exposure to a certain concentration beginning with the earliest stages, followed by lower concentration or by return to sea water; (*c*) early development in sea water, exposure to agent beginning at later stage (blastula, early planula).

The developmental stages of *Phialidium* are so susceptible to change in condition that some degree of developmental modification frequently occurs in sea water in the laboratory if conditions are not maintained near the optimum. For example, if the developmental stages remain too long in the container with large numbers of medusæ, differential inhibition of development results, the modifications bring similar to those induced by other means. Under these conditions there is a decrease in pH, resulting chiefly from the CO_2 production of the medusæ. There may also be some accumulation of other metabolic products and

[1] At the time these experiments were performed it was believed that the increase in H-ion concentration was the chief inhibiting factor in the acid experiments, but in the light of the recent work of Smith and Clowes ('24) it appears probable that the increase in CO_2 resulting from the addition of HCl to sea water is more important than the increase in H-ion concentration. In my experiments no attempt was made to determine CO_2 tension after addition of HCl.

some decrease in oxygen content. When development takes place in standing water several centimeters deep without renewal some degree of differential inhibition and modification usually occurs at the bottom while attachment and development of hydranth-stem axes proceed in the normal manner (Child, '25) on the sides of the container near the surface. Here also there is some decrease in pH at the bottom. When development takes place in a layer of water only a few millimeters deep no such differences appear and development is wholly or almost wholly normal if the material is otherwise in good condition. Occasional cases of modification usually occur even in the best material kept as nearly as possible under optimum laboratory conditions. Such modifications doubtless result from factors affecting particular eggs or spermatozoa before development or from poor condition of certain individuals.

Experiments with the medusæ as the inhibiting agents were performed by placing a number of the medusæ in small aquaria or bowls with the developmental stages and determining the pH colorimetrically from time to time.[1] The modifications produced by crowding, standing water etc., are exactly similar in character to those produced by the various other agents used. Some forty years ago Metschnikoff ('86) described as variations similar modifications in the development of *Mitrocoma*.

As will appear below, with the various agents and conditions used, developmental modifications result from the differential susceptibility of different body levels. All agents and conditions used inhibit development, the higher levels of the gradient being most inhibited. In the lower concentrations differential acclimation, or after return to sea water differential recovery may occur, the higher levels of the gradients acclimating or recovering more rapidly or more completely than the lower levels, as indicated by increased developmental activity.

[1] The work of Smith and Clowes ('24) makes it probable that in these experiments, as in those with HCl (see footnote, p. 177) the chief inhibiting factor is the CO_2 resulting from the respiration of the medusæ, rather than the increase in H-ion concentration brought about by the dissociation of carbonic acid formed from a part of the CO_2. The fact that the decrease in pH is rapidly reversed on shaking the water with air indicates that CO_2 production is responsible for it, but it is possible that other products of medusa metabolism may play some part in the inhibition of development.

Differential Inhibition in the Development of the Planula.

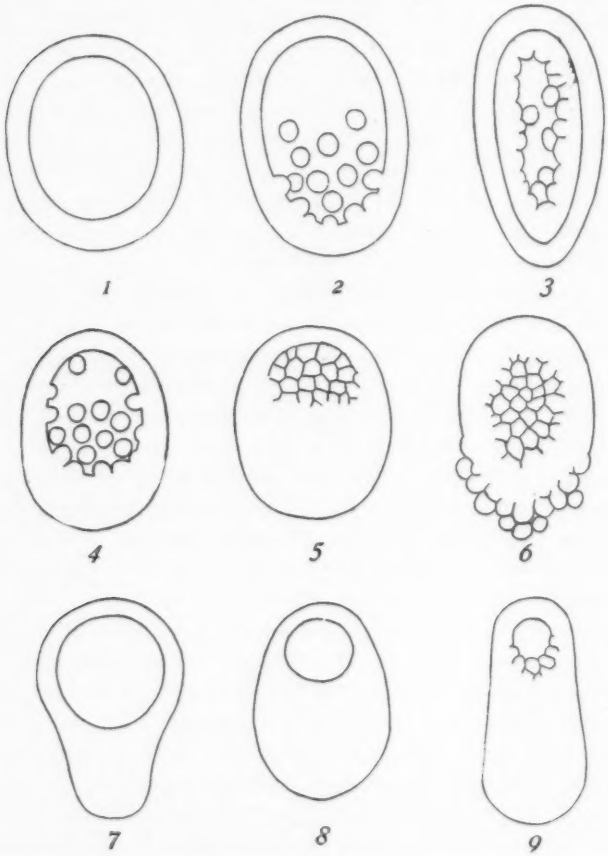
It is important to note first of all that there is no evidence of any specific effect on development of any agent used. The relations of the differential modifications to the gradient are similar for all agents, though the degree of differential action differs for different concentrations and for different agents, being greatest for LiCl, *i.e.*, in LiCl the high end of a gradient is relatively more, the low end relatively less inhibited than with other agents used. The high degree of differential action of lithium salts has also been noted by MacArthur ('24) in the modification of echinoderm development.

Since the modifications produced by the different agents are similar, detailed statements of the results obtained with each agent would involve much repetition and are omitted for the sake of brevity. The various degrees of modification are briefly described and data concerning their occurrence with different concentrations and agents are given.

The Lesser Degrees of Differential Inhibition.—In concentrations somewhat above those used for developmental modification the cleavage stages often separate into single blastomeres or collapse into irregular cell masses which sooner or later die, but in the concentrations used for modification of later stages, cleavage proceeds without appreciable alteration. Susceptibility is found to increase during early development and the lower susceptibility of cleavage stages as compared with later stages, together with the fact that more or less time is necessary for the inhibiting action to become evident, undoubtedly account for the absence of modification of cleavage with the concentrations used.

Figs. 1, 2 and 3 are diagrammatic outlines of blastula, immigration stage, and early planula as they appear under good conditions in the laboratory, and represent approximately "normal" development. Figs. 4–9 show the less extreme degrees of differential inhibition. These modifications are in general more frequent in material exposed to the lower concentrations from the beginning of development or to the higher concentrations from advanced cleavage or early blastula. They consist in different degrees of increase and extension of the basal thickening of the

blastula wall and of all degrees of reduction, even to complete obliteration, of the blastocœl by continued mass ingrowth of the thicker parts of the wall instead of, or in addition to immigration



of single cells. The thickened region is a solid cell mass, usually without distinct boundary between ectoderm and entoderm. Fig. 6 represents a condition observed thus far chiefly in LiCl in which the blastocœl is completely obliterated by cells and basal emigration is occurring.

In all these modified forms development is retarded and locomotion is much slower than normal. Many of these in-

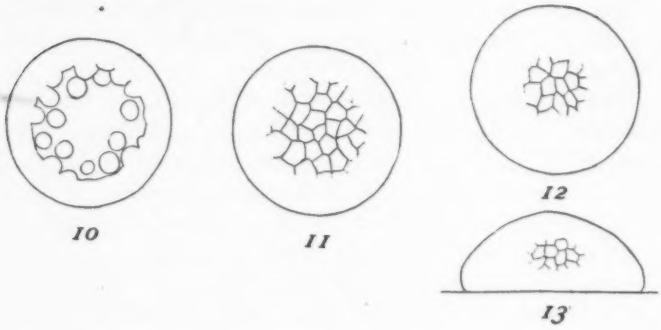
hibited forms advance slowly in contact with the bottom, being unable to support themselves free in the water, others revolve with little or no advance and some show reversal in the direction of locomotion (Figs. 6, 8). In these reversal of the susceptibility gradient and the reduction gradient (Child, '25) has also been observed.

These modifications represent an extension of basal characteristics and behavior toward the apical end, *i.e.*, a change of the more apical levels to the condition and behavior of the basal levels. The least degree of such modification consists merely in an increase of the basal thickening, an extension of the region of immigration toward the apical end and an increase in number of immigrating cells (Fig. 4). All gradations occur between this condition and forms in which almost the whole of the blastula wall is involved in immigration and ingrowth (Figs. 8, 9) and in some cases the cells of the basal region become so altered that they emigrate instead of immigrating (Fig. 6).

The differentially inhibited planulæ which result from earlier stages like Figures 4–9 are more or less elongated forms with excess of entoderm, often completely solid. Since they are usually not sufficiently active to swim free, locomotion takes place in contact with the bottom, in most cases with apical end in advance, but in some reversed, as noted above. As ciliary activity decreases these larvæ do not attach by the original apical end, but simply come to rest gradually with some meridian of the longitudinal surface in contact and more or less flattening occurs along this meridian. In the absence of differential acclimation or recovery development may go no further, or one or more stolon axes may develop slowly. In any case the body becomes enclosed in a delicate perisarcal secretion.

The More Extreme Degrees of Differential Inhibition.—These appear more frequently in the higher concentrations or in material subjected to inhibiting action from the beginning of development. They constitute a continuous series with the forms already described and are separated only for convenience. In material exposed to sufficiently high concentrations or degrees of action from the early cleavage stages, elongation of the blastula does not occur, the whole wall remains thick and immigration and mass ingrowth occur in all regions, resulting in a spherical solid

larva, usually without distinct boundary between ectoderm and entoderm. Figure 10 shows an early stage, Figs. 11 and 12 show later stages of these forms.



10 11 12 13

When exposure to inhibiting agents is begun only after the blastula stage is attained, polar elongation, basal thickening and immigration may occur in the agent. In the higher concentrations, however, immigration or thickening of the blastula wall gradually extends apically and may involve all regions of the wall, the blastocœl may be obliterated and the elongation may disappear completely, the extreme modification short of death being spherical solid, apparently completely apolar forms indistinguishable from those described above (Figs. 11, 12). In these cases there is actual regression so far as visible axial differences, elongation and basal thickening are concerned.

These spherical solid larvæ, whether they arise from earlier stages before elongation occurs, or from later elongated stages by regressive changes, are, so far as can be determined, apolar. Susceptibility gradient and reduction gradient are no longer present. They have lost the capacity for definitely directed locomotion and roll about on the bottom, being usually unable to support themselves free in the water. They show no definite attachment reaction, but gradually become quiescent and after movement ceases adhere by some part of the surface and become more or less flattened (Fig. 13). If conditions do not permit acclimation or recovery, no further development occurs, though they may live for two weeks or more. In short, the physiological differences constituting polarity have apparently been obliterated

and the pattern is no longer axiate, but merely surface-interior (Child, '24, pp. 57, 93).

DIFFERENTIAL ACCLIMATION AND DIFFERENTIAL RECOVERY.

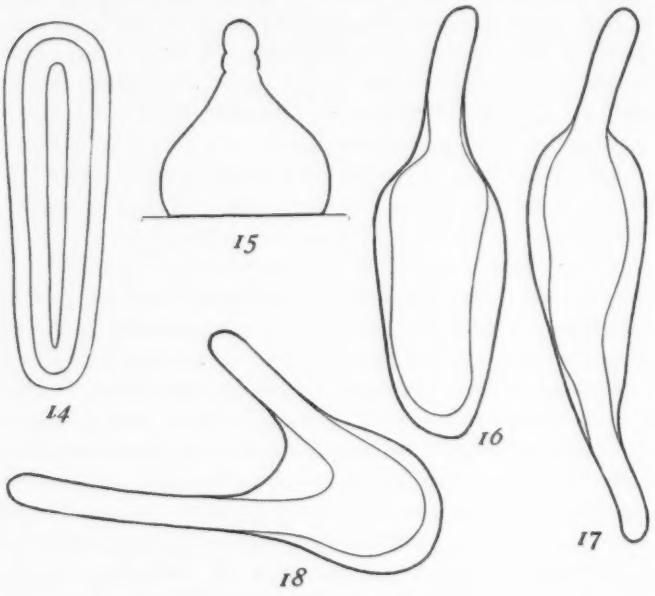
Under "normal" conditions the blastula develops into the elongated free-swimming planula (Fig. 14), which shows at first an apico-basal gradient, but later develops at the basal end a second gradient opposite in direction to the first, as described in the preceding paper (Child, '25). Later the planula attaches itself by the original apical end and the hydranth-stem axis develops from the original basal end, *i.e.*, from the secondary gradient (Fig. 15). As pointed out in the preceding paper, the hydranth-stem axis represents a new polarity arising by a process of budding from the original basal end of the planula.

In the lower concentrations of agents which produce at first some degree of differential inhibition without completely obliterating polarity, differential acclimation or acquirement of tolerance may gradually occur in the course of a few days with further modification of development. Similarly differential recovery may occur on return to well aërated sea water after temporary exposure to concentrations which produce differential inhibition. Within a certain range of differential inhibition the regions most inhibited acclimate or recover most rapidly or most completely, as is indicated by further development. Since differential acclimation and differential recovery are secondary changes which take place following a differential inhibiting action, it is evident that they can modify only the later stages of development.

The first indication of differential acclimation or differential recovery in differentially inhibited planulæ which still retain some degree of polarity is the outgrowth of a stolon from one or both ends. Fig. 16, apical stolon, and Fig. 17, apical and basal stolons, as seen from above, show characteristic forms. In the case of Fig. 18 the larva probably came to rest with the body bent upon itself and gave rise to a stolon from each end, though it is possible that one or both of these stolons represent new polarities. In these and later figures the heavy line indicates perisarc, the light line the outer surface of the cœnosarc.

As I have pointed out in an earlier paper (Child, '23), hydroid stolons represent somewhat inhibited physiological gradients or

reduced polarities and hydranth-stem axes can be easily transformed into stolons by inhibiting factors. In these planulæ stolons instead of hydranth-stem axes develop because in the earlier stages of acclimation or recovery a considerable degree of inhibition is still present. The apical region of the stolon axis is



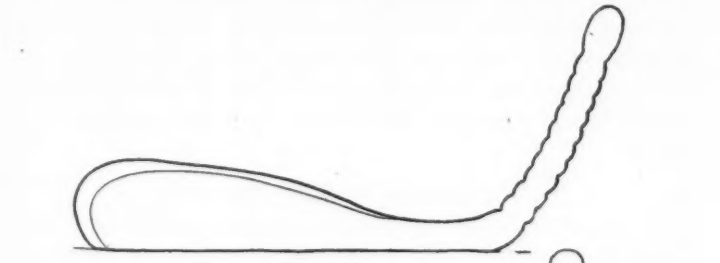
sufficiently active in relation to other levels to grow at their expense, but for the development of a hydranth-stem axis a higher metabolic rate is evidently necessary than for a stolon axis.

Figure 16 represents a larva in which the second gradient at the basal end did not develop before inhibition occurred, or was obliterated by the inhibition, while the original gradient was not wholly obliterated. In Fig. 17 and probably in Fig. 18 both gradients have persisted through the inhibiting action and both develop independently as stolons.

The further history of these stolon outgrowths depends on the degree of acclimation or recovery. When a certain degree of inhibition persists, *e.g.*, very commonly in acclimation, in which the material remains exposed to the agent, the stolon tips

continue to grow at the expense of the more basal levels, which are gradually resorbed. Such growth may continue for weeks and extend over many centimeters, being ended only by exhaustion of nutritive material. It is discussed more fully in a later section.

When the degree of acclimation or recovery is such that the gradients attain or approach their normal physiological condition, hydranth-stem structures develop. In differential acclimation and recovery this condition is usually attained only gradually, consequently the first outgrowths are almost invariably stolons and these transform later into hydranth-stem axes as in Fig. 19,



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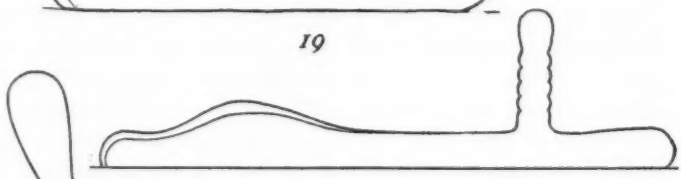

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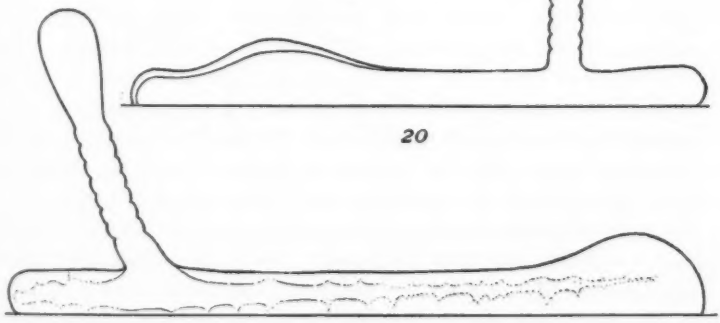

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or give rise by budding to such axes as in Figs. 20 and 21. In these figures the animals are viewed from the side, the original larval body and the stolons are in contact with the bottom and the hydranth-stem axes are more or less erect.

With the appearance of hydranth-stem axes the further growth

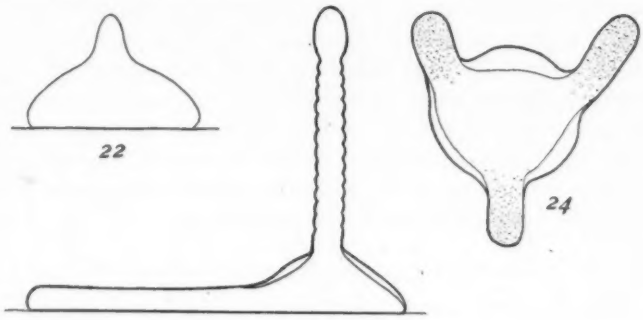
of the stolon is usually inhibited or retarded until the hydranth-stem axis attains sufficient length to permit some degree of physiological isolation of the stolon tip. Often the stolon serves as nutritive material for the developing hydranth and stem and may be completely resorbed. Fig. 19 shows a case in which such resorption is going on. The stolon, originally filling the perisarc, is much reduced and irregular in outline, while in the hydranth-stem axis the cœnosarc fills the perisarc. Moreover, the stolon cœnosarc, particularly in the most reduced regions, is almost transparent, while that of the hydranth-stem axis appears more granular and denser. By means of inhibiting conditions these hydranth-stem axes may be again transformed into stolon axes or inhibited to such an extent that they serve as material for the growth of stolon axes and are resorbed. With the approach of exhaustion also, the hydranth-stem axes usually undergo resorption, while the stolon axes persist and grow, at least for some days longer.

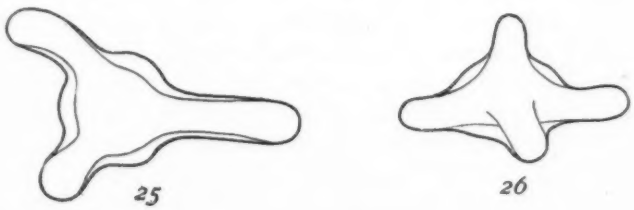
The Appearance of New Polarities.

As noted above, the spherical, apparently completely apolar forms undergo no further development unless some degree of acclimation or recovery occurs and then they apparently develop new polarities. Occasionally in cases of rapid recovery a hydranth-stem axis arises directly from the upper free surface of the flattened mass (Fig. 22). In the absence of landmarks the possibility cannot be absolutely excluded that this outgrowth represents the hydranth-stem axis of normal development. On the other hand, observation indicates that the spherical forms may come to rest with any part of their surface in contact and that their polarity has been completely obliterated. If this is true, the hydranth-stem axis developing from the upper surface represents a new polarity determined by differential exposure of free surface and surface in contact, probably involving differences in intake of oxygen or the giving off of CO_2 or both.

Figure 23 shows in side view a case of simultaneous development of two axes from a spherical apolar form. The axis arising from the upper surface is a hydranth-stem axis, that from the side, a stolon axis. Since these axes develop from a spherical, apparently apolar form, it seems probable that both represent

new polarities, the hydranth-stem axis being determined as a gradient by the greater respiratory activity occurring on the upper free surface, the stolon axis by some local region of greater activity on the margin.





Usually, however, acclimation or recovery occurs so slowly that the outgrowths which arise from these spherical forms are at first stolons and only later transform into, or give rise to hydranth-stem axes. In many of these cases there can be no doubt that new gradients or axes originate since three and not infrequently four stolons may arise from a single individual (Figs. 24–26, all viewed from above). These axes, or at least some of them, are evidently "adventitious," *i.e.*, they are determined by slight local chance differences among the cells, each stolon originating as a localized region in which the cellular activity is greater than in surrounding regions. This region begins to grow at the expense of other parts about it and as it grows the gradation in activity, which is at first about a center becomes an axial gradient as growth proceeds. Each outgrowing stolon is such a gradient

with high end at the tip. In Fig. 24 the basipetal gradient of cytolysis in lethal concentrations is indicated in the three stolons.

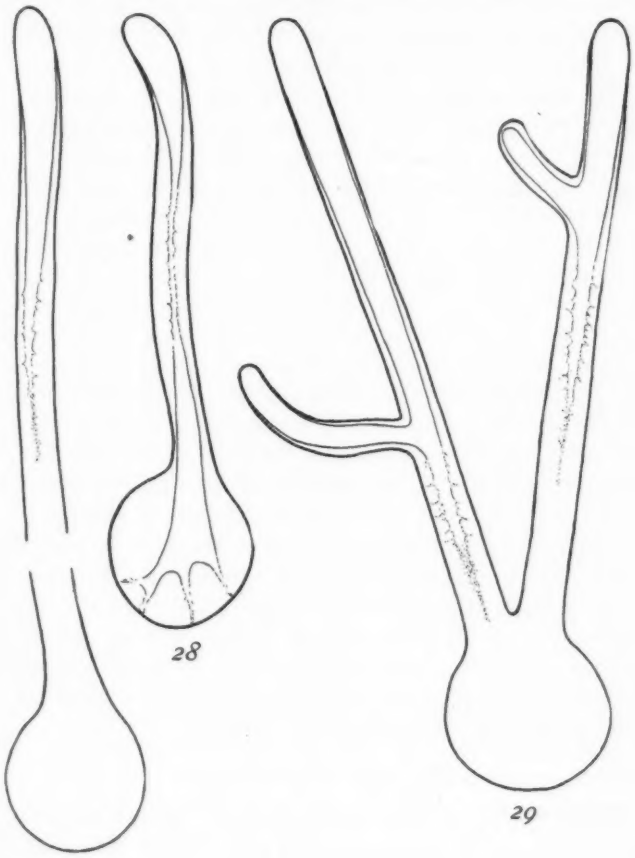
When acclimation or recovery proceeds far enough, the tips of such stolons sooner or later transform into hydranth-stem axes, like the stolon of Fig. 19, or hydranth-stem axes arise as adventitious buds from their upper surfaces, as in Figs. 20 and 21. In this way an individual which has been made apolar through differential inhibition may develop during acclimation or recovery several new stolon axes and each of these may later give rise to several hydranth-stem axes. On the other hand, if acclimation or recovery is only partial each stolon may continue to grow indefinitely as a stolon until exhaustion occurs.

The Growth and Separation of Stolons.

The continued growth of stolons under slightly inhibiting conditions shows certain features of interest, some of which were briefly described in an earlier paper (Child, '23) as observed in stolons arising from hydroid colonies in consequence of inhibiting conditions. The stolons arising from the inhibited developmental stages grow at the expense of other parts and in time the whole substance of the inhibited larva may be used in the growth of the stolon or stolons arising from it, leaving only the empty perisarc in the position of the larval body and an empty perisarcal tube connecting this with the stolon. Fig. 27 shows a case in which an inhibited form gave rise to a single stolon, which finally used up the whole larval body in its growth. At the stage shown the tip of the stolon had reached a point several centimeters distant from its point of origin, only a small part of the perisarcal tube connecting stolon and larval body being shown in the figure. After using the substance of the larval body, the stolon tip continues to grow at the expense of its more basal levels. The cells of the tip remain in good condition, while those of the basal end become shrunken and transparent and undergo atrophy. In this way the cœnosarc of the stolon becomes shorter and shorter until only a small mass of tissue remains.

Figure 29 shows a case in which an inhibited form gave rise to two stolons near together. At the stage shown they have used the whole larval body and then their own substance to such an extent that they are entirely separate from each other, except for

the perisarcal tubes, and each has branched. In the later history of this case the cœnosarc of the larger lateral branch and that of the main stolon from which it arose became separated and each pursued its own course, the tip continuing to grow at the expense of more basal regions. The shorter lateral branch on the other main stolon is undergoing resorption at the stage figured and later was completely resorbed. Occasionally a growing stolon leaves a part of the larval body behind as in Fig. 28. This



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apparently occurs when the stolon is not very active and the gradient is therefore short. Under such conditions the tip is apparently able to obtain nutrition only from regions within a short distance. The region in which atrophy occurs evidently represents approximately the limit of this distance.

In the earlier stages of this process of growth during starvation the development of hydranth-stem axes from such stolons may be induced by providing optimal conditions, but in later stages this transformation occurs less frequently and with the approach of exhaustion, but before stolon growth ceases, all attempts to induce transformation have thus far failed.

This continued growth of the stolon tip at the expense of the larval body and later of its own substance is an interesting case of a more active region, maintaining itself at the expense of less active regions. Whether the atrophy of the less active regions represents primarily a more rapid autolysis or some other process, it is clearly evident that the most active region, the high end of the stolon gradient, succeeds in using the substance of all other levels, not only for maintenance, but for growth. Such stolons may grow over a distance of 10–15 centimeters before exhaustion occurs. Doubtless each stolon level lives at the expense of levels below it and sooner or later becomes a source of nutrition to levels above it in the gradient. This relation may mean simply that less active regions undergo autolysis more rapidly and that the more active regions are able because of their activity to use the products of autolysis in maintenance and even in growth.

A similar relation between different levels of the axial gradient appears in other cases of reduction by starvation. For example, in planarians and other forms undergoing reduction the proportions approach those of younger animals because the higher levels of the polar gradient, particularly the head region, maintain themselves to some extent at the expense of lower levels and so undergo reduction less rapidly than those.

Experimental Records.

The uniformity of result makes it entirely unnecessary to give the records of all experiments in full. A few characteristic records are given in Table I. as examples. Other records differ from these only as these differ from each other, *i.e.*, in degree and

rate of differential inhibition, acclimation or recovery, according to the conditions of the experiment. The various forms, normal and modified, are indicated in Table I. by abbreviations as follows:

B. Normal blastula (Figs. 1, 2).
IB. Differentially inhibited blastula (Figs. 4–9).
Ap. Spherical or flattened apolar form (Figs. 10–13).
EP. Early planula (Fig. 3).
LP. Late planula, elongated (Fig. 14).
IP. Differentially inhibited planula, shorter, thicker than normal, with excess of entoderm.
H. Hydranth-stem axis developing directly from basal end of planula attached by apical end; "normal" hydroid development (Fig. 15).
NH. Hydranth-stem axis developing directly from apolar form (Figs. 22, 23).
StH. Hydranth-stem axis developing indirectly by transformation of, or budding from a stolon axis (Figs. 19–21).
St. Stolon axis (Figs. 16–18, 24–29).
Dd. Dead.

Each series of Table I. consisted of one hundred or more eggs or early embryos. All different forms observed were recorded at each examination and many drawings were made. In the table the relative frequency of the different forms is roughly indicated by enclosing in parentheses the designations of those forms which were present only in relatively small numbers (approximately 20 per cent. or less). The designations not so enclosed represent the characteristic forms of the series at the time indicated. As indicated at various points in the table the solutions were sometimes diluted after inhibition had occured, particularly when the appearance of the material indicated that development was not likely to proceed farther in the concentration originally used. Such dilution made acclimation possible or hastened it. The series given are characteristic. Frequent repetitions during the same and different summers showed nothing essentially different from the data presented.

Taking Series 4 I. as an example, the Table shows that in KCN, *m*/25,000 the forms after one day were inhibited blastulæ and

TABLE I.

Series.	Controls	2	4 I	4 II	3 I	3 II	9	8 III	5
Agent	water	KCN	KCN	KCN	HCl	water 2 days HCl	LiCl	LiCl	medusæ 2 days
Concentration		m/20,000	m/25,000	m/50,000	pH 7.4+	pH 7.2	m/80	m/160	pH decreased to 7.5
Days after beginning of experiment									
1	B EP	(B) IB Ap	(B) IB Ap	EP IP	IP Ap		IB (Ap) diluted 3/4	IB Ap	IB
2						EP (LP)		IP Ap	(EP) IP (Ap) medusæ removed; water
3		IP Ap returned to water	(IP) Ap	LP IP (Ap)	St Ap		(IP) Ap	IP Ap (St)	
4	H(StH LP)	(IP) Ap Dd	(IP) Ap diluted 1/3		St(StH Ap) diluted 1/3	(LP) St Ap diluted 2/3			
5		(IP Ap) Dd				St (Ap)		(StH St) Ap Dd	IP Ap
6	H (StH)		(IP) Ap	H StH St	StH St				St (Ap)
8							StH St Ap		(StH) St
9			(StH) St Ap						
12			NH StH (St Ap)						

apolar forms with a few normal blastulæ; after four days chiefly apolar forms with a few inhibited planulæ; solution now diluted one third; after nine days chiefly outgrowing stolon axes and apolar forms with a few stolon axes giving rise to hydranths; after twelve days chiefly new hydranth-stem axes arising directly from previously apolar forms and hydranth-stem axes arising from stolon axes, with a few stolon axes bearing no hydranths and a few apolar forms still remaining.

Modification of Development in *Gonothyræa*.

A few experiments with HCl and KCN, exposure beginning with the early planula after emergence from the gonophore, were sufficient to show that the modifications resulting from differential inhibition and from differential acclimation and recovery are the same in *Gonothyræa* as in *Phialidium*. To decrease in pH the susceptibility of *Gonothyræa* is less than that of *Phialidium*. For example, HCl, pH 7.4 has very little inhibiting effect on *Gonothyræa*, the planulæ usually attaching in the normal manner and giving rise to hydranth-stem axes without stolon outgrowths. Series 3 I. of Table I. shows that this pH is strongly inhibitory for *Phialidium*. For obliteration of polarity in *Gonothyræa* a concentration of HCl giving pH 7–6.8 was found to be necessary and as acclimation occurred, stolon axes developed and even gave rise to hydranths. As might be expected from these results crowding with the medusæ had little or no effect on development within the range used for *Phialidium*.

Discussion.

The differentially inhibiting effect of the agents on the blastula and early planula stages is sufficiently evident. In the normal blastula the region of basal thickening and of immigration represents the low end of the apico-basal gradient (Child, '25) and the effect of the agents is to bring higher levels of the gradient into a condition in which they behave like the basal region. In the extreme cases (Figs. 10–12) in which polarity is apparently obliterated, the whole blastula wall, including even the apical pole, behaves like the basal region. Taking this behavior as a criterion, it appears to be true that the whole blastula is reduced to the physiological level of the basal region. Evidently such a

change in condition is differential with respect to the axis: the apical region is most, the basal region least affected. When this reduction of all regions to the same physiological level is complete, the physiological gradient is obliterated and the fact that such forms show no indications of polarity until new gradients are determined in them is additional evidence for the conclusion that the gradient constitutes the polarity.

The fact that the basal region usually shows growth and thickening far in excess of the normal may seem at first glance to conflict with the interpretation of these modifications in terms of differential inhibition, but, as a matter of fact such overgrowth is a consequence of the differential inhibition. The basal region is less inhibited than the apical, consequently it is more active relatively to the apical region in the inhibited than in the normal forms. This relatively greater activity enables it to obtain a larger proportion of the available nutritive substance and so to grow to a larger size, though more slowly than in normal development. Up to a certain degree the excess and extension of basal thickening increases with the decrease in apical, as compared with basal activity. But with more extreme degrees of differential inhibition all levels become more and more alike and more basal as regards physiological condition and differences of growth and development disappear, as in the spherical apolar forms. The complete disappearance of these differences and the reduction of the larva to a spherical apolar condition are more likely to occur when the inhibiting factor has acted from the early stages of development and has obliterated the gradient before the differences in behavior of ectodermal and entodermal regions have been established.

As pointed out above, actual reversal of the physiological gradient occurs in some cases, the reversal being indicated by reversal of orientation in locomotion and reversed susceptibility and reduction gradients. In such cases the differential inhibiting action of the agent is such that the original apical region is reduced to a lower level of activity than the original basal region. Since such reversal occurs only after basal thickening has begun, it may result in a great increase of that thickening. The cases of emigration of cells from the basal region (Fig. 6) suggest that the polarity of these cells, *i.e.*, the differences between the exterior

and the interior pole, have also undergone reversal, so that instead of immigrating into the blastocœl the cells emigrate.

It may be noted in passing that the less extreme degrees of differential inhibition (Figs. 5–9) show interesting resemblances to the exogastrulæ and related forms such as solid blastulæ of the echinoderms, which also result from differential inhibition (MacArthur, '24). In both the hydrozoan and echinoderm modifications the entoderm—and in echinoderms the mesenchyme—is greatly increased at the expense of ectoderm, because the lower levels of the gradient are normally less active and therefore less susceptible and less inhibited than the higher levels. Actual reversal of the susceptibility gradient may also occur in both hydrozoan and echinoderm. The cases of emigration of cells (Fig. 6) correspond most closely to the exogastrulæ.

Spek ('18) has maintained that gastrulation is due to difference in the degree of swelling of internal and external parts of the cells concerned and that lithium salts and some other agents determine exogastrulation by reversing these differences. Whether or not this interpretation be correct, the differential action of the agents shows clearly the same relation to the gradient in both hydrozoan and echinoderm.

That the inhibition, even though it does not obliterate polarity, interferes with the usual sequence of events in later stages is shown by the fact that when acclimation or recovery occurs, the outgrowth may be apical (Fig. 16) or both apical and basal (Fig. 17), that is, the stolon may arise from the primary gradient or from both primary and secondary gradient and probably also from the secondary alone (Fig. 19). These differences undoubtedly depend on differences in stage of development at time of exposure and on degree of inhibition. The development of both apical and basal stolons indicates that under the inhibiting conditions the two ends have become independent of each other.

The conditions determining the "adventitious" polarities in the acclimation and recovery of apolar forms (Figs. 24–26) are obviously accidental for no two individuals are alike as regards localization and time of appearance of the different axes. Evidently these axes originate as local areas of slightly greater activity in some region or regions of the mass. Some of them may represent persisting traces of the original polarity or

polarities, but some of them are certainly, and all of them may be new axes. The fact is of interest that in such forms adventitious stolon-axes may arise from any part of the free surface, but the direct origin of a hydranth-stem axis has been observed only from the most exposed portion farthest from the surface in contact (Figs. 22, 23).

The fact that the stolon axes usually appear first in acclimation and recovery and only later transform into, or give rise by budding to hydranth-stem axes confirms the conclusion of an earlier paper (Child, '23). In that paper it was shown that apical regions of various hydroid colonies can be transformed into stolon axes by slight degrees of inhibition or depression and that the stolons can again give rise to hydranth-stem axes with acclimation to, or recovery from the action of the agent. Such apical transformations do not represent reversals of polarity, but rather simply a depression of the gradient to a lower physiological level. In Lund's experiments on *Obelia* with electric current (Lund, '21) the position of the piece with respect to the electrodes merely determines whether the gradient arising at a given end shall be more or less inhibited and develop as a stolon or whether it shall attain the higher physiological levels characteristic of the hydranth-stem axis. Since axes, either stolon or hydranth-stem, very commonly arise from both ends of pieces without action of the electric current, it is evident that the current is not necessary for the determination of these polarities, even though it does determine their character as stolon or hydranth-stem axes and may, when acting, assist in determining the gradient at one end or the other of the piece. In any case the effects of the current are in no way specific as regards determination of the two sorts of axes, for, as I have shown, essentially the same results can be obtained with various chemical and physical agents in both hydroid colonies and developmental stages. Apparently all that is necessary to transform a hydranth-stem gradient into a stolon-gradient is a slight degree of inhibition, and the reverse transformation is accomplished by environmental changes in the reverse direction. In Lund's experiments with *Obelia* the anode apparently acts to some extent as an accelerating, the cathode as an inhibiting agent.

Physiologically the stolon does not represent simply the basal

end of the hydranth-stem axis, but is itself an axis, a gradient, as has been demonstrated in various ways. Like new hydranth-stem axes, it originates from a bud and the high end of its gradient constitutes a growing tip instead of a hydranth. At the stolon tip synthesis of protoplasm, growth and cell division apparently keep pace with each other as in the growing tip of a plant, so that differentiation does not occur, but when the tip is raised to a higher physiological level by change in external conditions it loses the capacity for unlimited growth and differentiates sooner or later into a hydranth.

The demonstration that the stolon is a somewhat inhibited axis or gradient throws light on many otherwise puzzling features of the behavior of isolated pieces of hydroids and hydroid colonies. The stolon gradient may be inhibited, either by external conditions or by its nearness to a dominant hydranth region (Child, '15, pp. 91–2). Consequently a stolon axis may arise at a cut surface which is near vigorous hydranth regions and it may also arise under conditions which prevent formation or maintenance of hydranths. Pieces in deep standing water may produce stolons, those in shallow, or frequently changed water hydranth-stem axes. Pieces may produce first stolons, then hydranth-stem axes or *vice versa*, with slight differences in conditions of culture.

Three conclusions based on the evidence presented in this and earlier papers of this series and on the work of Lund and others are important in this connection: first, the stolon as well as the hydranth-stem represents an axis, a polarity, a gradient; second, the stolon gradient represents primarily lower levels of physiological activity than the hydranth-stem gradient and reversible transformation in either direction is possible and does not necessarily involve reversal of polarity: third, hydranth-stem or stolon axis may be determined according to conditions by the quantitative differential or local action of environmental factors independently of their specific constitution. These conclusions afford a physiological basis for the interpretation of data at hand and for further investigation and control of polarity.

Summary.

1. Differential inhibition of planula development can be brought about in the hydrozoan, *Phialidium gregarium*, by KCN, LiCl, ethyl urethane HCl (probably CO_2) and by the presence of the medusæ (CO_2). Resulting modifications range from slight increase and extension of basal thickening of blastula wall followed by development of slightly modified planulæ to spherical, solid, apparently apolar forms. In these immigration and mass ingrowth are increased and take place from all parts of the wall, and further development occurs only if a new polarity arises.

2. In differential acclimation in low concentrations of inhibiting agents and differential recovery after return to water further development of persisting axes may occur or new polarities may arise through differential exposure of the surfaces of apolar forms or through "adventitious" localization of regions of greater activity. In rapid acclimation or recovery such axes may develop directly as hydranth-stem axes, but usually they are at first still more or less inhibited and develop as stolons and their tips transform later into hydranth-stem axes, or such axes arise by budding from their free surfaces.

3. The stolon represents a polarity, a gradient, at a lower level of physiological activity than the hydranth-stem axis. Transformation of hydranth-stem into stolon results from inhibition, of stolon into hydranth-stem from acceleration; neither involves necessarily a reversal of polarity.

REFERENCES.

Child, C. M.

'15 Individuality in Organisms. Chicago.

'23 The Axial Gradients in Hydrozoa. V. Biol. Bull., XLV.

'24 Physiological Foundations of Behavior. New York.

'25 The Axial Gradients in Hydrozoa. VI. Biol. Bull., XLVIII.

Lund, E. J.

'21 Experimental Control of Organic Polarity by the Electric Current. I. Jour. Exp. Zoöl., XXXIV.

MacArthur, J. W.

'24 An Experimental Study and a Physiological Interpretation of Exogastrulation and Related Modifications in Echinoderm Embryos. Biol. Bull., XLVI.

Metschnikoff, E.

'86 Embryologische Studien an Medusen. Wien.

Smith, H. W. and Clowes, G. H. A.

'24 The Influence of Carbon Dioxide on the Velocity of Division of Marine Eggs. Amer. Jour. Physiol., LXVIII.

Spek, J.

'18 Differenzen im Quellungszustand der Plasmakolloide als eine Ursache der Gastrulainvagination sowie der Einstülpungen und Faltungen der Zellplatten überhaupt. Kolloid-chem. Beihefte, IX.

DIRECT AND AFTER EFFECTS OF CHANGES IN MEDIUM DURING DIFFERENT PERIODS IN THE LIFE HISTORY OF *UROLEPTUS MOBILIS.*

I. EFFECTS OF BEEF EXTRACT.

LOUISE H. GREGORY.

In 1905 Woodruff [1] as a result of his experiments with salt stimulation stated, "the conclusion seems to be justified that a given stimulus produces different effects at different periods in the life cycle." For the past two years while working on the problem of the nutrition of *Uroleptus mobilis*, I have had the opportunity of testing the sensitiveness of the protoplasm of different ages and find that so far as the division rate is concerned, the nature of the response to beef treatment and in all probability to treatment with potassium phosphate and other salts can be predicted if the age of the protoplasm is known.

The material used in these experiments was taken originally from the stock of Professor Calkins at Columbia, and the same series numbers were kept. From time to time conjugations occurred in the stock cultures at Barnard and new series were started in which case the letter *B* has been added to the series number to distinguish it from the Columbia series of the same number but possibly not of the same ancestry. In the present paper the results are given of experiments with beef extract only. In a later paper, the experiments with potassium phosphate and other salts will be reported.

A. The Direct Effects of Beef Feeding.

Professor Calkins[2] has cultivated *Uroleptus mobilis* for the past six years on a hay-flour medium, made "by boiling 100 mgs. chopped hay with 130 mgs. flour in 100 cc. Great Bear Spring water for ten minutes and diluting this when 24 hours old with equal parts of spring water." Woodruff and Baitsell [3] found that a .025 per cent. solution of Liebig's beef extract was a favorable medium for certain infusoria, and after experimenting with solutions of varying strengths, this percentage has been used in

all of the following experiments with beef extract. A .05 per cent. solution of beef extract made up with Great Bear Spring water and a 24-hour-old solution of flour water of the same strength as in the normal hay-flour medium of Calkins, were used in equal amounts. This resulting medium was a solution of the most favorable beef strength and exactly similar to the normal medium except that the beef solution had been substituted for the hay.

In conducting the experiments one individual was taken from each of the five lines of the control series and placed in the beef flour medium. Both series were kept under the same conditions of light and temperature. They were transfered to fresh medium at the same times and differed only in the hay or beef element of the medium. As in all similar experiments the rate of division was taken as the indication of the vitality of the protoplasm.

Discussion of Experiments.

(*a*) *Direct Response of Young Protoplasm to Beef Extract.*

Table I. shows the results of feeding very immature series with the beef-flour medium. Six young series of five lines each were tested for their reaction to the substitution of beef extract for the hay in normal medium and in every case there was a lowering of the vitality indicated by the slowing of the division rate. The amount of the depression varied from 1.8 to 9.6 divisions in ten days for each line of the series. Every young line responded in the same way indication that in this period which in the six cases extended from the 5th to the 40th generation, the protoplasm was in a condition in which the normal metabolic processes were easily and immediately upset and the vitality lowered.

Table I.

Effects of Beef Feeding during the Period of Immaturity.

Series No.	Age in Gen.	Av. No. Divisions per Line in 10-day Periods, Beef Series.	Av. No. Divisions per Line in 10-day Periods, Control Series.	Amt. of Depression in Division Rate per Line in 10 Days.
82....	17–24	11	21.6	9.6
97....	11–34	17.6	20.8	3.2
98 B..	17–40	20	24.8	4.8
99 B..	11–30	15.2	17.6	2.4
100 B..	11–25	13.2	15.0	1.8
101 B..	5–23	13.6	18.4	4.8

(*b*) *Direct Response of Older Protoplasm to Beef Extract.*

Table II. shows the results of feeding *Uroleptus* with the beef-flour medium when the protoplasm was older than in the experiments shown in Table I. Series 97, 98*B*, 99*B*, 100*B*, 101*B*, all of which had been depressed in the beef-flour medium when tested earlier in their life history, now are stimulated by the treatment. Series 97 during the 60–75th generations showed an increase in ten days of 6.8 divisions per line over the control series. The stimulating effect of the beef was seen again though to a less degree in the 167th generation and even in as late a period as the 225th generation, the beef series maintained a slightly higher rate of division than the control. Series 98*B* depressed in the 40th generation, was stimulated in the 75th and 122d generations and at both times, the division rate exceeded that of the control. This same increase it vitality was observed in varying degrees in Series 99*B*, 100*B*, and 101*B* each one having been previously depressed by the beef treatment. In addition to the above experiments, three other series were tested whose reaction to beef in their earlier history was not known. Series 72 was in its 50th generation when it was obtained from Columbia. A series was treated with beef for 40 consecutive days and throughout the entire period from the 50th to the 140th generation, the stimulating effects of the beef were apparent. Even in the 180th generation the division rate of the beef series was equal to that of the control. Series 81 during the 56th–84th generation, was treated with beef for 20 consecutive days. During the first ten days the division rate was slightly less than that of the control. In the second ten period, however the beef series showed a more rapid division rate than the control. Series 95 was treated for 30 consecutive days, from the 50th–102d generation and at no time was there any evidence of stimulation. When the series was again tested in the 160th, 190th and 210th generations, the beef series responded with a much higher division rate than the control. Thus all eight series in their later life history responded to beef treatment with an increase in the vitality as indicated by a quickened division rate. The age at which this increase appears varies as would be expected. Series 99, 100, 101, all divided more rapidly in the beef medium at an early age, one as young as

27 generations. Series 72, 97, 98 showed the stimulating effects of the beef about the 60th–75th generation. Series 81 and 95 failed to respond to the beef until a still later age and might be considered slow maturing series. Series 95 gave indications of a long period of immaturity, showing distinct depression in the beef as late as the 102d generation. Beef apparently is a depressant in the early generations and a stimulant in later periods of the life history.

TABLE II.

THE EFFECTS OF BEEF FEEDING DURING A LATER PERIOD IN THE LIFE HISTORY.

Series No.	Age in Gen.	Av. No. Divisions per Line in 10-day Periods, Beef Series.	Av. No. Divisions per Line in 10-day Periods, Control Series.	Amt. of Increase in Division Rate per Line in 10 Days, Beef Series.
72....	58–79	19.8	18.4	1.4
"	79–100	20.6	19.2	1.4
"	100–125	17.0	14.0	3.0
"	125–140	16.8	9.2	5.6
"	180–200	13.6	13.6	0.0
81....	56–68	16.0	16.4	−0.4
"	68–84	16.0	14.0	1.4
95....	50–70	18.0	18.0	0.0
"	70–84	14.0	16.0	−2.0
"	84–102	12.0	18.0	−6.0
95....	160–175	19.6	16.6	3.0
"	190–210	22.4	17.8	4.6
"	210–225	20.8	15.7	5.1
97....	60–75	21.6	14.8	6.8
"	167–185	16.2	15.2	1.0
"	235–254	11.4	11.2	.2
98 B..	75–97	19.0	17.8	1.2
" ..	122–136	16.0	14.0	2.0
99 B..	40–60	17.4	14.4	3.0
" ..	75–90	13.4	12.6	.8
" ..	95–105	11.6	11.8	.2
100 B..	27–58	17.4	16.4	1.0
" ..	90–105	13.0	11.6	1.4
101 B..	39–64	14.2	11.0	3.2
" ..	80–102	14.0	13.8	.2

(c) Direct Response of Old Protoplasm to Beef Extract.

Table III. shows the results of feeding old series with the beef-flour medium. Eleven experiments were carried out on protoplasm varying in its age from the 210th to the 370th generation

and in every case the result was one of depression and a lowering of the vitality. Series 61 taken from Columbia stock in the 250th generation was depressed to such an extent in the beef-flour medium that its division rate was cut from 16.4 divisions in ten days to 7.6 divisions in ten days and during the next ten day period the series died while the control was dividing normally. Series 69 was treated for 40 days when the protoplasm was from 175–335 generations old, and at no time did the division rate of the beef series equal that of the control, the average rate per line for each ten day period being 9.8 divisions while that of the control series was 14.5. divisions. Series 95 failed to show indications of age until the 230th generation when the beef series divided in ten days on an average of 3.2 divisions less than the control. When this same series was again placed in the beef-flour medium in the 310th generation, the division rate fell to 6.0 divisions per line less than that of the control. Series 97 was tested for its reaction to beef six times in its life history and after the initial depression in the period of youth, the vitality was not lowered until the 260th generation. From this age on, there was a definite slowing of the division rate for the beef, flour series. Series 61, 69, 77, 88 were all old series when taken from the Columbia stock so there was no opportunity to test them when younger. In their old age however, they gave the expected response to the beef-flour treatment.

A comparison of the results of these three sets of experiments indicates that the protoplasm of *Uroleptus* responds in a definite manner to treatment with beef according to its age. The very young individual apparently does not adjust its self to the new medium, its metabolic processes are retarded and its vitality lowered. At a later stage in its life history it is able at least to adjust itself to the change in medium and to maintain a vitality as great as that of the control. Then appears an age when it is not only able to adjust its self to new conditions but is stimulated by them and shows an immediate definite increase in its division rate. Finally with age there is again a lack of adjustment indicated by the lowering of vitality and slow division rate. If the series is very old the substitution of the beef is sufficient to cause the death of the race.

TABLE III.

EFFECTS OF BEEF FEEDING DURING THE PERIOD OF OLD AGE.

Series No.	Age in Gen.	Av. No. Divisions per Line in 10-day Periods, Beef Series.	Av. No. Divisions per Line in 10-day Periods, Control Series.	Amt. of Depression in Division Rate, Beef Series.
61.....	360–370	8.8	16.4	7.6
69.....	275–335	9.8	14.5	4.6
72.....	200–225	11.2	12.0	.8
"	225–236	11.4	12.0	.6
77.....	210–230	13.8	16.2	2.4
88.....	255–270	7.6	8.8	1.2
95.....	230–243	10.6	13.8	3.2
"	280–290	6.2	8.0	1.8
"	305–310	0.8	6.8	6.0
97.....	260–270	5.4	7.2	1.8
"	270–283	2.6	6.6	4.0

No definite time can be set for the appearance of maturity or the onset of old age. If a definite favorable response to the beef treatment is an indication of maturity, it may occur as early as the 30th or as late as the 102d generation. For the average series it would appear about the 60th generation which agrees with the statement of Calkins that the period of youth or immaturity covers about the first 60 generations. Old age usually shows its self about the 225th generation but it may appear as early as the 200th or as late as the 260th generation. Old age and immaturity outwardly are alike in their response to beef treatment. They also seem to agree in their relative duration. If the period of youth is prolonged as in series 95 the period of old age is likewise longer than that of the average. Series 95 lived 350 generations and showed indications of old age during the last 120 generations, Series 97 matured in about the average time of 60 generations and showed evidences of age only in the last 40 generations, the race dying in the 300th generation.

B. The After Effects of Beef Feeding on Protoplasm of Different Ages.

In a number of experiments after a series had been kept in the beef-flour medium for ten days, one individual from each of the five lines of the series was put back into normal medium and the division rate of the new series compared with that of the series continued in the beef-flour medium.

Three young series after ten days in the beef medium were transfered to normal medium and in every case the vitality was increased to such an extent that it exceeded that of the beef series and equaled that of the normal control series. In other words the depression while in the beef medium was followed by a stimulation on the return to normal medium.

Four mature series were treated in the same manner some twice and others three times, and in one experiment only was there any indication of a stimulus when the series was transfered from the beef to normal medium. In five experiments the transfer was followed by a distinct lowering of the division rate and in three other experiments, there was no change in vitality as indicated by the division rate.

Finally series 95 and 97 were tested twice each in their old age and in all four experiments the transfer to normal medium was followed by a distinct increase in vitality.

Here again is seen the similarity in the behavior of immature and worn out protoplasm. In both cases there is an immediate depression of the life processes when treated with the beef, but this depression disapears on removal to normal conditions and in some cases there follows a period of higher vitality, the real effect of the beef being only delayed in its appearance. On the other hand the mature individuals respond immediately with a more rapid division rate which falls when the stimulus is removed and the medium is again normal. Immature protoplasm and protoplasm of old age are apparently not able to adjust themselves quickly to changes in the environment. They are at once depressed in the new condition but overcome the depression when back in normal medium and often show a quickened vitality. Mature protoplasm is like a healthy muscle that is always in tone, ready to respond to a stimulus and needs no time

for adjustment to the changed conditions, thus the effects appear at once and would not be expected to continue when the cause has been removed. It may be concluded that beef is always more or less of a stimulant to Uroleptus but the time when the stimulating effects are apparent varies with the age. Mature races will respond at once. Immature and old series are at first depressed and show the stimulating effects only after they are once more in their normal environment.

TABLE IV.

AFTER EFFECTS OF BEEF FEEDING.

Series No.	Age While in Beef.	Av. No. Divisions per Line in 10-day Periods, Transfered Series.	Av. No. Divisions per Line in 10-day Periods, Beef Series.	Effect of Change from Beef-Flour to Normal Medium.
82...	17–24	18.0	5.4	Stimulation
99 B.	11–30	16.8	16.2	"
101 B.	24–34	15.2	13.8	"
98 B.	75–97	15.6	16.0	Depression
" .	122–136	12.6	16.0	"
" .	158–170	11.2	12.2	"
99 B.	40–60	15.6	15.6	No effect
" .	80–105	11.0	11.6	Depression (slight)
" .	105–120	10.8	10.2	Stimulation (slight)
100 B.	37–58	14.2	14.2	No effect
" .	90–105	10.6	13.0	Depression
101 B.	39–64	14.0	14.0	No effect
95...	280–290	8.2	7.8	Stimulation
" ...	305–310	5.0	0.8	"
97...	235–255	8.0	7.4	"
" ...	270–84	8.2	2.6	"

SUMMARY.

The response of *Uroleptus mobilis* to treatment with a beef-flour medium varies with the age of the protoplasm. Both young and old series are immediately depressed in the beef medium but recover their vitality and may show a greater division rate than that of the control series when transferred to the normal hay-flour medium. Mature series on the other hand are stimulated when placed in the beef medium. The stimulation however is not

continued when the series is returned to the normal environment. Beef seems to be an immediate stimulant if the protoplasm is mature, and a delayed stimulant if the series is young or old, the effect being seen only after transference to normal hay-flour medium.

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REFERENCES.

1. **Woodruff, L. L.**
'05 Jour. Exp. Zoölogy, Vol. 2, No. 4, p. 585.
2. **Calkins, G. N.**
'19 Jour. Exp. Zoölogy, Vol. 29, No. 2, p. 121.
3. **Woodruff and Baitsell.**
'11 Jour. Exp. Zoöl., Vol. 11, p. 135.

EFFECTS OF CARBON DIOXIDE.

Effects of Different Tensions of Carbon Dioxide on Certain Orthoptera (Grasshoppers).[1]

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Introduction.

Much attention has recently been given to the physiological effects of carbon dioxide upon mammals, while comparatively little information in this respect seems to exist for invertebrates. Because of this lack of data it seemed desirable to study the physiological significance of this gas for certain insects. The present paper is based upon a quantitative study of the resistance of the grasshopper to different tensions of carbon dioxide noting particularly the muscular movements displayed under the influence of the gas, the rate of recovery from its effects, and respiratory changes in an enclosed space, as measured by carbon dioxide and oxygen changes.

I wish to thank Doctor J. H. Bodine, under whose direction the work was done, for suggestion of the problem and for inspiring criticism throughout.

Material and Methods.

It has been shown in a recent paper by Doctor Bodine (1) that grasshoppers have a relatively high rate of carbon dioxide output and this fact suggested that they might be favorable material for determining some of the effects of carbon dioxide on insects. In carrying out the following experiments two species of *Melanoplus* were used: *Melanoplus differentialis* and *Melanoplus femur rubrum*.

Inasmuch as the methods employed are so varied, individual descriptions are given under each of the several experiments.

[1] Data in this paper taken from a thesis submitted to the faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Master of Science.

In order to prevent confusion of connotation of the words used in describing the endpoints in the experiments the two following terms are defined:

1. First movement—designates the first reflex, *i.e.*, crawling or stirring of the animal after it has been removed from the influence of the gas.

2. Recovery—indicates that the animal has returned to a normal state.

Experiments.

1. *Effects of Pure Carbon Dioxide.*

The animals used in these experiments, *Melanoplus differentialis* and *Melanoplus femur rubrum*, were raised entirely under laboratory conditions.

Table I.

Showing Variation in Time to First Movement after Short Exposures to Pure Carbon Dioxide.

Animals:	*Melanoplus* (*differentialis* and *femur rubrum*) Adults									
Exposure Minutes:	1				2			5		
Species:	*M.dif.*	*M.f.r.*	*M.f.r.*	*M.f.r.*	*M.dif.*	*M.f.r.*	*M.f.r.*	*M.f.r.*	*M.f.r.*	*M.dif.*
Sex:	♀	♀	♀	♀	♀	♀	♂	♂	♂	♂
Number of Exposure.	Minutes to First Movement.									
1	7	4	4	5	4	4	2	6	11	13
2	4	5	4	7	3	2	2	8	10	5
3	5	4	5	8	2	2	2			
4	6	5	4	7						
5	5	5	Aver. 5.2		Aver. 2.6			Aver. 9		

Adults and nymphs of both sexes were placed separately in airtight tubes connected with a Kipp generator. The carbon dioxide was made by the action of hydrochloric acid on calcium carbonate, passed through concentrated sulphuric acid, pyrogallol and distilled water, thence into tubes containing the animals, and

finally, into distilled water and out to the air. The animals were exposed to the steam of pure carbon dioxide for definite lengths of time ranging from one minute to twenty-four hours. They were then removed and the time to the first movement noted. The character of the movements from the beginning of the exposure until the animals had recovered was also observed.

Table I. shows the range, especially in shorter exposures, in minutes to the first movement. The variations shown are due perhaps to individual differences. From an examination of this table it may also be seen that when the animal is subjected to the influence of pure CO_2 for one minute and then removed, the time to the first movement varies from four to eight minutes with an average of 5.2. With two minutes exposure the time to the first movement is from two to four minutes with an average of 2.6. With five minutes exposure the variation is greater, being from five to thirteen minutes with an average of nine. Above five minutes exposure, that is from ten minutes to about three hours, there is less variation. With five hours exposure, there may be no response, and if any, the movements start immediately upon removal from the gas.

TABLE II.

SHOWING TIME AND RATE OF THE FIRST MOVEMENT AFTER EXPOSURE TO THE GAS FOR DEFINITE PERIODS OF TIME.

Exposure, Time in Minutes.	Time to First Movement; Average—Minutes.	Rate of First Movement, per Minute Exposure.
1	5.1	5.1
2	5.1	2.5
5	4.4	0.88
10	6.3	0.63
15	5.0	0.33
20	3.8	0.19
22	6.0	0.27
25	5.0	0.20
30	4.0	0.13
40	5.6	0.35
60	7.9	0.13
90	1.5	0.016
120	7.93	0.066
180	21.5	0.12
320	6.2	0.017
5 hours	Immediate	0

Table II. shows the average time in minutes to the first movement and the calculated rate per minute exposure to the gas. The data presented in this table give results for about two

hundred animals exposed for varying periods. It is evident from an examination of this table and from Fig. 1, which shows the same results graphically, that the rate of first movement per minute exposure is apparently inversely proportional to the length of time the animals are exposed.

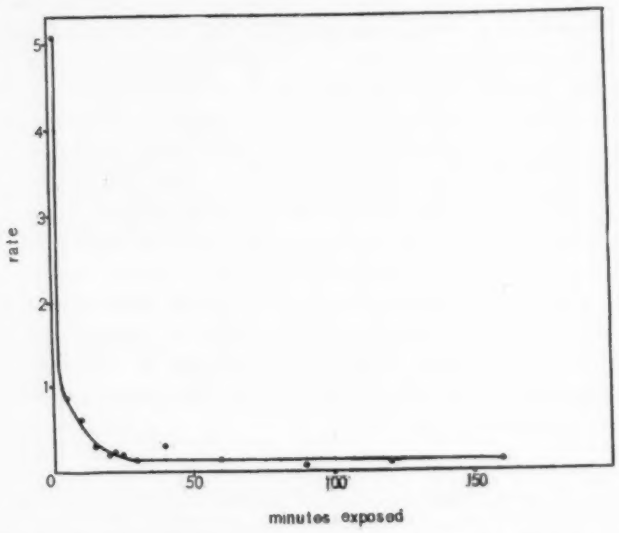

FIG. 1. Abscissa: The length of time in minutes the animal is kept under the influence of pure CO_2. Ordinate: Rate in minutes per minute exposure to the gas, of the first movement. (For further description see text.)

From Table II. and Fig. 1, it may also be seen that up to about thirty minutes exposure to the gas the change in rate of the first movement is rapid; above this there is no perceptible change.

It seems that the length of exposure to carbon dioxide, when above one hour, has an effect on the time to the first movement. Below this time limit, variations may be considered as due to individual differences. Perhaps a thousand determinations would be necessary to establish each point on a perfect curve. However, individual differences vary within limits and the data from the experiments performed are sufficient to determine the trend of the curve, as is given in Fig. 1.

In order to determine the relation between the first movement

and recovery, animals of the same species, age and sex as above, were exposed to a stream of pure carbon dioxide for definite lengths of time ranging from one minute to five hours. The animals were then removed from the tubes, the time to the first movement as well as the time until the animals recovered was noted.

TABLE III.

SHOWING RELATION BETWEEN FIRST MOVEMENT AND RECOVERY AFTER EXPOSURE TO THE GAS FOR DEFINITE PERIODS.

Exposure, Minutes.	*A* First Movement, Minutes.	*A'* Rate of First Movement per Minute Exposure.	*B* Recovery, Minutes.	*B'* Rate of Recovery per Minute Exposure.
1.....	3.03	3.03	9.65	9.65
4.....	1.9	0.46	7.7	1.9
10.....	6.6	0.66	11.7	1.2
15.....	5.0	0.33	11.0	0.73
20.....	4.5	0.22	19.7	0.98
40.....	5.6	0.14	14.2	0.35
60.....	10.6	0.17	37.5	0.62
2 hours ..	13.27	0.11	39.0	0.32
3 hours ..	21.5	0.11	34.0	0.19
5 hours ..	—	—	—	—

Table III. gives the average values obtained from 45 animals, and 37 exposures to the gas and 136 endpoints, several animals being used in each exposure.

Generally, it may be said, from the data of Table III. and Fig. 2, curve *B*, that the longer the exposure, the longer the time required for the animal to recover. As for the time relation between the first movement and final recovery, there is sufficiently little variation as seen in Table III., columns *A* and *B*, to assume that within the limits of one to forty minutes exposure, the animals are affected, physically or chemically, in a similarly reversible manner. However, the longer exposures take a relatively longer time for recovery.

Columns *A'* and *B'* of Table III. and curves *A'* and *B'* of Fig. 2, show the rate of first movement and of recovery, per minute exposure, to follow the same type of curve. This perhaps signifies that reversibility is of the same nature in all cases. Variation in points determined, preventing regular curves are attributed to individual physiological differences in response.

Some idea of the striking characteristics of the movements of the grasshoppers produced during and after they are under the influence of carbon dioxide, may be derived from the following paragraph.

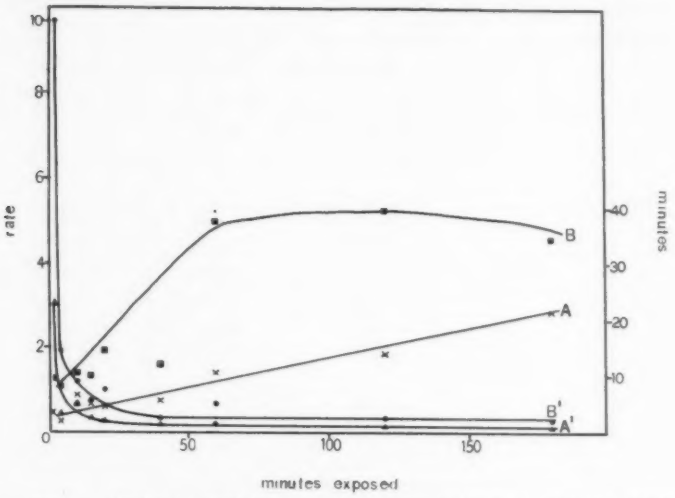

FIG. 2. Abscissa: Length in minutes of exposure to the gas. Ordinates at left, rate p. min. exposure. At right, time in minutes.

Curve *A* = Time in minutes to the first movement.
Curve *B* = Time in minutes to the recovery.
Curve *A'* = Rate of first movement per minute exposure.
Curve *B'* = Rate of the recovery per minute exposure.
(For further description see text.)

When an animal is subjected to the gas, it undergoes violent reflexes in all appendages—at times, the abdomen, due to stimulated respiratory movements, curls and uncurls; however, some animals make no movements. The period before asphyxiation occurs is less than one minute, usually about twenty to thirty seconds. After asphyxiation the animal becomes stiff or limp, depending upon the individual, and apparently lifeless, although some may exhibit slight reflexes. They remain in this condition until removed from the gas. In longer exposures some animals secrete an excessive amount of saliva and the antennæ droop. The salivary secretion may occur at any stage or upon removal from the gas. The first movements after removal from the gas

vary with the individual. Usually there are slight reflexes, or quivers and tremblings of the legs, the large hind legs often being the first to move. After these reflexes have continued for a few seconds, becoming more and more pronounced, the legs become either stiff or limp. This latter condition may be followed shortly by a return of the reflexes and after longer exposure the reflexes may become violent. When the reflexes have subsided the animal begins to crawl feebly, slowly recovers its strength and appears normal. After shorter exposure the response differs, the first movement after removal from the gas may be a crawl or a hop, followed by normal movements.

Often it is impossible to compare the resistance of the animals when time alone is considered with regard to an arbitrary endpoint. However, if the first movement is taken as the result of a stimulus brought about by the interchange of gases when a new equilibrium is being established it may be a true endpoint for comparing resistances to different length exposures to carbon dioxide. As for a comparative resistance of the animals to carbon dioxide taking into consideration the character of the movements, as numerical data alone do not suffice, it may be said that: (1) *Melanoplus differentialis* is more resistant than *Melanoplus femur rubrum;* (2) nymphs of both species are more resistant than adults, they do not succumb so readily and recover more completely in a shorter length of time. Adults undergo the most violent reflexes, secrete more saliva and take the longest time to recover fully; (3) molting or freshly molted animals succumb most readily and recover very quickly from reversible doses, perhaps due to the softness of the chitin; (4) no sex differences were observed; (5) starvation for twenty-four hours has no appreciable influence on the recovery from the effects of the gas.

2. *A Measure of the Change in Percentage of Carbon Dioxide and Oxygen, Due to Respiration or Some Interchange of Gases between the Animals and the Air in an Enclosed Space.*

The following readings are based upon *Melanoplus differentialis*, nymphs and adults of both sexes being used.

A Haldane gas analysis apparatus was used for determining the per cent. of oxygen and carbon dioxide. The type of container

used was as follows: A 160 cubic centimeter bottle, with a neck 1.5 centimeter in diameter was fitted with a rubber stopper carrying a 10 centimeter long capillary tube reaching within one centimeter of the bottom of the bottle. The outer end of the capillary tube was fitted with a piece of heavy rubber tubing carrying a clamp. This tube was fitted over the capillary of the Haldane apparatus when the air to be analyzed was taken into the burette of the apparatus.

In making a determination, an approximately known volume of carbon dioxide (made as described above) was transferred from a nitrometer tube to the container filled with atmospheric air and open to prevent increased pressure. The container was then closed and a known amount of air, about 8 cubic centimeters was withdrawn from it into the Haldane apparatus—previously filled with nitrogen made by removing oxygen and carbon dioxide from atmospheric air. The percentages of oxygen and carbon dioxide were then determined from this sample. The stopper was withdrawn, the animal introduced and the bottle quickly closed. Probable error at this point is negligible because of the slow diffusion of carbon dioxide. Since the air in the bottle was put under slight negative pressure in the first analysis, it was thought that some outside air might enter. However, immediate analysis showed no change great enough to affect the results of the experiments. Not more than three to five readings were made on each bottle so that negative pressure produced would not influence the normal reaction of the animal.

By the above method, using the Haldane apparatus, determinations are accurate to 0.02 per cent. Thus the results after taking into consideration individual differences, should be comparable to at least 0.1 per cent.

TABLE IV.

SHOWING PERCENTAGE CHANGES IN CO_2 AND O_2 DURING EXPOSURE TO THE GAS FOR DEFINITE PERIODS OF TIME.

Exposure.	Per Cent. Absorbed by Animal.		Per Cent. Given Off from Animal, O_2.	Per Cent. Difference Given Off from Animal, O_2.
	CO_2.	O_2.		
5–11	1.84	0.95	2.38	1.43
27–36	3.99	2.43	2.78	0.35
48–73	5.68	1.01	0.99	0.02
3 hours	10.03	1.53	0	—

It was found that with an original range of 28 to 32 per cent. carbon dioxide and 12 to 13 per cent. oxygen there was but a slight difference in the final composition of the air within the bottle. Table IV. is a compilation of data within this range. It

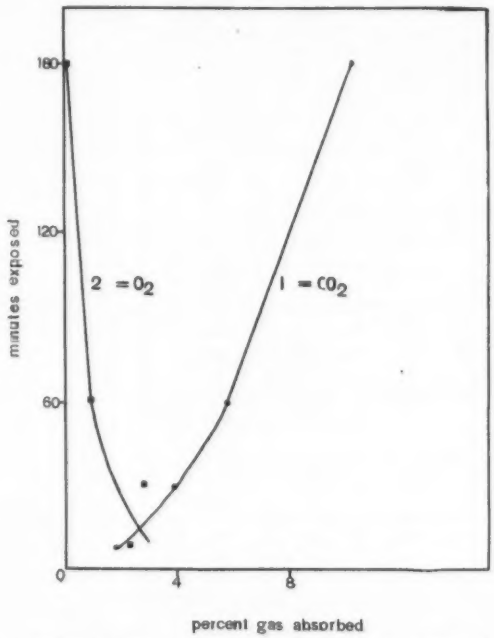

FIG. 3. Abscissa, Curve 1: Per cent. gain of CO_2 in tissues of the animal. Curve 2: Per cent. loss of O_2 from tissues of the animal. Ordinate: Length of exposure in minutes. (For further description see text.)

is evident from this table and curve 1 of Fig. 3 that carbon dioxide is at first rapidly, then gradually, absorbed by the animal. An initial gain in oxygen as shown in the same table and curve 2 of Fig. 3 may be due to the slight negative pressure, some of the oxygen diffusing out of the tracheal tubes of the animal. However, later it is reabsorbed.

Table V. gives data for varying percentages of carbon dioxide combined with varying oxygen percentages. Column 3 of this table gives the ratio of carbon dioxide to oxygen in each case. In column 4 the lengths of time in minutes the animals were exposed to the gases are noted and in columns 5 and 6 the attendant

TABLE V.

SHOWING CHANGES IN O_2 AND CO_2 DURING VARIOUS PERIODS OF EXPOSURE TO VARYING TENSIONS OF O_2 AND CO_2.

Original Per Cent.		Ratio CO_2/O_2.	Exposure, Minutes.	Absorbed by Animal—CO_2.	Given Off by Animal—O_2.
CO_2.	O_2.				
7.95	18.32	0.44	6	0.64	−1.58
			27	0.43	0.51
			105	1.49	1.11
			143	1.44	0.92
29.82	13.30	2.24	10	3.63	1.1
			20	2.14	1.86
			73	4.71	1.60
29.39	12.73	2.31	11	0.35	0.54
			30	−0.42	1.29
			65	2.01	−0.69
			238	7.26	0.22
			252	11.98	−4.32
32.77	13.23	2.48	10	2.22	−1.81
			36	2.58	2.00
30.28	10.28	2.94	30	0.5	−2.00
			180	3.51	−2.05
			5.5 hrs.	2.57	3.71
35.94	11.75	3.07	10	1.31	−0.27
			49	8.89	−3.67
41.09	7.80	5.27	10	4.78	3.01
			27	5.09	−0.20
			48	8.22	−1.30
51.88	8.64	6.01	5	4.57	2.57
			29	7.88	4.78
			74	9.82	1.78

changes in carbon dioxide and oxygen percentages are given. Figs. 4 and 5 show these results graphically. Following the changes in per cent. of oxygen and carbon dioxide as shown in this table as well as the curves in Figs. 4 and 5, it appears that with a constant percentage of oxygen, provided it is within the limit of 7 to 18 per cent., the rate, per minute exposure, of carbon dioxide absorption increases with increasing carbon dioxide percentages. In addition, it may also be said that with 75 to 100 per cent. carbon dioxide no measurable interchange of gases takes place.

As previously stated, oxygen may at first be given out by the animal; however, in some cases it is absorbed. This absorption

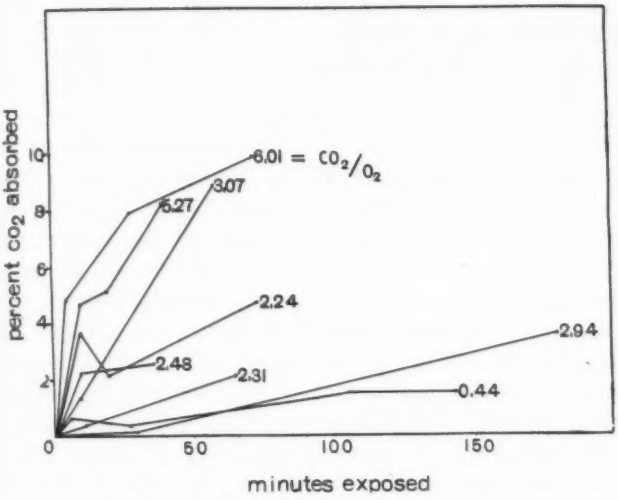

FIG. 4. Abscissa: Length of exposure in minutes. Ordinate: Per cent. CO_2 absorbed by the animal. (For further description see text.)

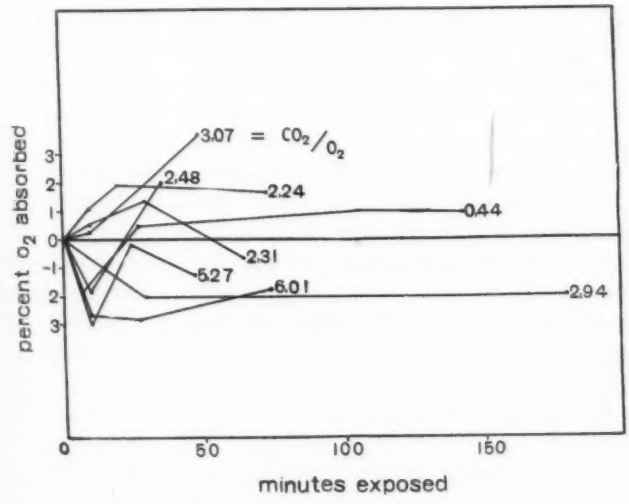

FIG. 5. Abscissa: Length of exposure in minutes. Ordinate: Per cent. O_2 absorbed by the animal. (For further description see text.)

or giving out of oxygen is found to occur within the first 30 minutes of exposure to the gases, but as the period of exposure increases, the gas is reabsorbed.

Discussion.

Respiration in insects depends upon the supply of air in the system of tracheæ or tubes ramifying through the body. Equilibrium is maintained by muscular movements of the abdomen as well as by diffusion of gases and, for this purpose, eight separate segments of the abdomen, on either side in a ventro-lateral position, bear a spiracle or small tracheal opening through which the air in the tubes is periodically changed.

The relation between the respiratory and circulatory systems of insects differs from that of the vertebrates and is of such an arrangement that the blood seems to have little to do with the transportation of oxygen and carbon dioxide except in a purely mechanical way. In the grasshopper, the body fluid is kept circulating by means of a tubular heart lying along the dorsal side of the body cavity and as there are no blood vessels, the fluid directly bathes the tissues. Thus, it is by means of the minute tracheal vessels of the respiratory system that the gases are carried into the body and diffuse through the delicate tracheal membranes into the blood and tissue cells.

In a qualitative work on the influence of gases on the respiratory movements of grasshoppers, Walling (2) found that hydrogen inhibits the respiratory movements, then the animal partially revives and will continue to live in the hydrogen atmosphere for as long as four days. In oxygen the animal may be either active or dormant, depending upon the individual and, finally, dies from lack of food. Carbon monoxide reacts similarly to carbon dioxide and the animal will recover after longer exposures to the gas. As for carbon dioxide she found respiratory movements to cease within twenty to thirty seconds, but they returned when the animals were removed to fresh air. They could endure long exposures.

The characteristic behavior exhibited by the animals in the present experiments under the influence of carbon dioxide is perhaps due to physical changes in the tissue cells. It is known that carbon dioxide and oxygen may exist in solution in their body

fluid. Consequently with an excess of carbon dioxide it is quite probable that much of it is absorbed by the tissue cells. Jacobs (3) has demonstrated the easy permeability of the cell membranes to carbon dioxide and in studying the effect of carbon dioxide on protoplasmic viscosity of *Paramecia* and of *Arbacia* eggs, he found that shorter exposures to the gas cause a great decrease in viscosity while longer ones cause an increase and within limits both are reversible (4). Hence changing viscosity of the protoplasm of the grasshopper muscle cell attended by liquefaction or gelation may produce the limpness or stiffness of the animal.

There may also be a chemical explanation of the effects of the gases on the animals. Carbon dioxide, by going into solution in the blood increasing the hydrogen ion concentration, or by acting in an undissociated state, may stimulate the nerve ganglia in the abdominal segments causing breathing movements, thus producing an effect similar to that caused by stimulation of the respiratory center in mammals as demonstrated by Hooker, Wilson and Connett (5) and by Scott (6).

Another theory is suggested by the work of Kidd (7) wherein he attributes the resting stage in moist seeds to a narcotic action of carbon dioxide. Perhaps this idea may be correlated with certain data on insects. Buddenbrock and Rohr (8) working with the walking stick, *Dixippus morosus*, found that the rate of breathing movements is affected by the carbon dioxide content of the air. With increasing concentrations from 0 to 1 per cent. the rate of breathing suddenly increases, this is followed by a decrease; again at 10 to 15 per cent. there is a gradual increase; at 25 to 30 per cent. a decrease follows and at 35 to 40 per cent. respiratory movements cease. In the present paper there was found to be little or no change in the carbon dioxide content of an enclosed space containing an animal, when the original content was from 75 to 100 per cent. carbon dioxide. This excess carbon dioxide undoubtedly produces a deep narcosis in the grasshopper.

It may be of interest to note that experiments have been carried out by the author using a modified Van Slyke (9) method for determining the carbon dioxide content of insect blood. It seems that the carbon dioxide content of grasshoppers' blood is lower than that of mammalian blood and is approximately 30 cubic centimeters CO_2 per 100 cubic centimeters of whole blood,

although sufficient data have not as yet been collected to state this exactly.

It would be of some importance to know the proportion of carbon dioxide that exists in the blood as free carbonic acid since the hydrogen ion concentration of the blood would be affected by varying carbon dioxide tensions unless a strong buffer system were present. With this idea in mind, a colorimetric hydrogen ion concentration determination on the same principle as that described by Cullen (10) was made and seems to indicate that the normal blood of *Melanoplus differentialis* is near a pH of 6.1.

Summary.

1. It appears that exposures of *Melanoplus differentialis* and *Melanoplus femur rubrum* to a stream of pure carbon dioxide for various lengths of time up to about 30 to 40 minutes produce reversible effects. Further, the time to the first movement and to recovery after short exposure seems to be independent of the length of exposure. Longer exposures to the gas require longer periods for recovery.

It follows that the rate of the first movement and of recovery per minute exposure to the gas, up to 30 to 40 minute exposures, decreases rapidly. Above this limit the decrease in rates is imperceptibly gradual or remains constant until the exposure is sufficiently long to kill.

2. At 5 to 24 hours exposure to pure carbon dioxide, the animals are irreversibly affected and finally die. They may or may not exhibit reflexes upon removal from the gas.

3. Characteristic behavior, due to the influence of carbon dioxide, is exhibited.

4. There are three interdependent factors influencing the exchange of gases between the animal and the air in an enclosed space, namely, the original per cent. of carbon dioxide; the original per cent. of oxygen; the original ratio of carbon dioxide to oxygen.

Literature Cited.

1. **Bodine, J. H.**
'22 Jour. Exp. Zoöl., XXXV., 47.

2. **Walling, Eulalia V.**
'06 Jour. Exp. Zoöl., III., 621.

3. **Jacobs, M. H.**
'20 Amer. Jour. Physiol., LIII., 457.

4. **Jacobs, M. H.**
'22 Amer. Jour. Physiol., LIX., 451.

5. **Hooker, D. R., Wilson, D. W. and Connett, H.**
'17 Amer. Jour. Physiol., LIII., 351.

6. **Scott, R. W.**
'18–'19 Amer. Jour. Physiol., XLVIII., 43.

7. **Kidd, F.**
'15–'17 Proc. Roy. Soc. London, 89-*B*, 136.

8. **Buddenbrock and Rohr.**
'22 Zeit. f. Alleg. Physiol., Bd. XX., 111.

9. **Van Slyke, D. D.**
'17 Jour. Biol. Chem., XXX., 347.

10. **Cullen, Glenn E.**
'21 Jour. Biol. Chem., LII., 501.